CHAPTER THREE

The Molecular Toxicology of Chemical Warfare Nerve Agents

Kimberly D. Spradling[1] *and* James F. Dillman III*

Contents

1. Introduction 112
2. Nerve Agent Toxicity 114
   2.1. Route of exposure 114
   2.2. Acute toxicity of OP nerve agents 114
3. Current Medical Countermeasures to Nerve Agent Toxicity 116
4. Global Molecular Screening Approaches Enable the Identification of Molecular Mechanisms of Toxicant Exposure 117
   4.1. Genomics 117
   4.2. Proteomics 118
   4.3. Metabolomics 120
   4.4. Bioinformatic tools 120
5. Global Molecular Techniques Support the Three-Phase Model of Nerve Agent Toxicity 121
6. Global Molecular Techniques Provide Evidence for Non-AChE Mechanisms of OP Nerve Agent Toxicity and Reveal Secondary Effects of Exposure 123
   6.1. Oxidative stress 124
   6.2. Mitochondrial dysfunction 125
   6.3. Apoptosis 126
   6.4. Inflammation 128
   6.5. BBB dysfunction 130
   6.6. Repair and recovery 132
7. Future Directions in the Molecular Toxicology of Nerve Agents 134
8. Conclusions 135
Acknowledgments 138
References 138

Cell and Molecular Biology Branch, US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, Maryland, USA
*Corresponding author. Tel.: +1 410-436-1723; Fax: +1 410-436-1960
E-mail address: james.dillman@us.army.mil

[1] Current address: Department of Genetics, Texas Biomedical Research Institute, San Antonio, Texas, USA.

Advances in Molecular Toxicology, Volume 5
ISSN 1872-0854, DOI: 10.1016/B978-0-444-53864-2.00003-7
© 2011 Elsevier B.V.
All rights reserved.

Material may be protected by copyright law (Title 17, U.S. Code)

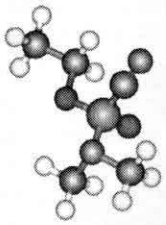
Abstract

Chemical warfare nerve agents continue to be a threat to both military personnel and civilian populations. Organophosphorus nerve agents irreversibly inhibit the enzyme acetylcholinesterase, resulting in accumulation of high levels of the neurotransmitter acetylcholine (ACh) at muscarinic and nicotinic receptors. This accumulation of ACh induces clinical symptoms including myosis, difficulty in breathing, convulsions, seizures, and can result in death. Current medical countermeasures for treating nerve agent intoxication increase survival if administered rapidly after exposure but may not fully prevent brain injury. The downstream neurological damage induced by nerve agent exposure is not well characterized. Researchers are now utilizing molecular approaches to understand the molecular pathways involved in nerve agent-induced brain injury, with the goal of developing treatment strategies that are effective when administered after the onset of seizures and secondary responses that lead to nerve agent-induced brain injury.

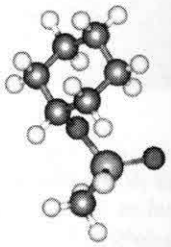
1. Introduction

Chemical weapons have been used for centuries to incapacitate or kill the enemy in times of war. Arrows dipped in scorpion and serpent venoms were used in the late Stone Age and are among the earliest documented chemical weapons [1]. Since then, chemical weapons have evolved and have been successfully used on the battlefield and in terrorist attacks [1–5]. The most toxic chemical warfare agents known to date are organophosphorus (OP) nerve agents, which were first developed in the 1930s by German scientists who were originally tasked with synthesizing more potent pesticides using organic phosphoric acid esters [3,6] (Figure 1).

Chemist Dr. Gerhard Schrader synthesized the first German nerve agent (or G-agent) in 1936. It was the cyanide-containing compound ethyl dimethylamidocyanophosphate, which was later named tabun (GA) [3]. After the realization that tabun was extremely toxic to humans and therefore unsuitable for use as a plant protectant, the German army immediately began producing it as an agent of chemical warfare. Further studies of this toxic compound led Schrader and his colleagues to synthesize the fluorine-containing nerve agent sarin (GB; isopropyl methanefluorophosphonate) in 1938, which was determined to be more than 10 times as toxic as tabun. The most toxic G-agent, soman (GD; *o*-pinacolyl methylphosphonofluoridate), was then synthesized by Dr. Richard Kuhn in 1944. Cyclosarin (GF; cyclohexyl methylphosphonofluoridate) was later synthesized by Schrader and colleagues in 1949 [3]. Although the German army produced large quantities of these nerve agents, there is no evidence indicating that they used them as chemical weapons.



GA (tabun)



GF (cyclosarin)

Figure 1 Structures

Following the disc
J. F. Newman first dis
Laboratories of Britair
of organophosphate c
these agents were toc
were further evaluat
Porton Down in the
in this family of ner
methylphosponothioa
agents, all of the V-ag
skin and other surface
times more toxic tha
chemical warfare ner

Despite the develo
in the first half of the
has changed in recent
term "weapon of ma
tion of chemical we
chemical weapons gl

Material may be protected by copyright law (Title 17, U.S. Code)

ue to be a threat to both military person-
ophosphorus nerve agents irreversibly
ase, resulting in accumulation of high
choline (ACh) at muscarinic and nicotinic
nduces clinical symptoms including myo-
ons, seizures, and can result in death.
treating nerve agent intoxication increase
exposure but may not fully prevent brain
amage induced by nerve agent exposure
are now utilizing molecular approaches
s involved in nerve agent-induced brain
atment strategies that are effective when
es and secondary responses that lead to

d for centuries to incapacitate or kill
ped in scorpion and serpent venoms
are among the earliest documented
emical weapons have evolved and
ield and in terrorist attacks [1–5]. The
n to date are organophosphorus (OP)
d in the 1930s by German scientists
esizing more potent pesticides using
ure 1).
nesized the first German nerve agent
anide-containing compound ethyl
was later named tabun (GA) [3].
emely toxic to humans and therefore
nt, the German army immediately
nical warfare. Further studies of this
olleagues to synthesize the fluorine-
opyl methanefluorophosphonate) in
ore than 10 times as toxic as tabun.
o-pinacolyl methylphosphonofluor-
nard Kuhn in 1944. Cyclosarin (GF;
) was later synthesized by Schrader
the German army produced large
is no evidence indicating that they

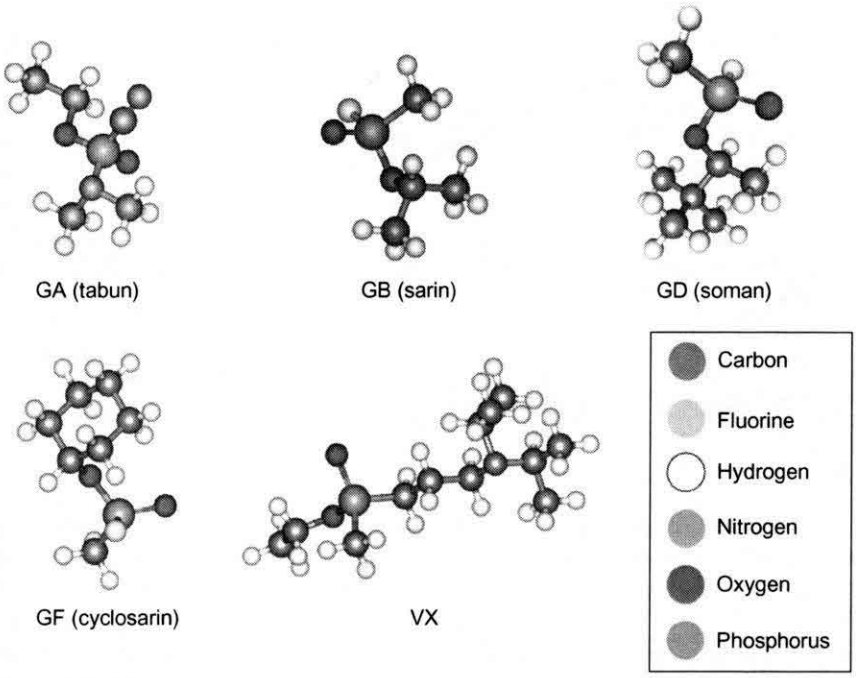


Figure 1 Structures of G-series and V-series organophosphorus nerve agents.

Following the discovery of the G-series agents, Drs. Ranajit Ghosh and J. F. Newman first discovered the V-agents in 1952 at the Plant Protection Laboratories of Britain's Imperial Chemical Industries while studying a class of organophosphate compounds. As with tabun, scientists soon realized that these agents were too toxic to be used as plant protectants. These agents were further evaluated at the British Armed Forces research facility at Porton Down in the United Kingdom, where the most studied compound in this family of nerve agents, VX (*o*-ethyl-s-[2(diisopropylamino)ethyl] methylphosponothioate), was synthesized in 1954. Unlike the G-series agents, all of the V-agents are persistent, meaning that they can remain on skin and other surfaces for long periods of time. The V-series agents are ~10 times more toxic than the G-agent sarin and are the most highly toxic chemical warfare nerve agents synthesized to date [3,7].

Despite the development and military proliferation of chemical weapons in the first half of the twentieth century, the worldview on these weapons has changed in recent history. In 1991, UN Resolution 687 established the term "weapon of mass destruction" and called for the immediate destruction of chemical weapons in Iraq and the eventual destruction of all chemical weapons globally. Further, the Chemical Weapons Convention

Material may be protected by copyright law (Title 17, U.S. Code)

of 1993 banned the production, stockpiling, and use of chemical warfare agents. Despite these extensive efforts to prevent their use, OP nerve agents still remain a threat to soldiers in time of war as well as to the civilian population in the event of a terrorist attack, such as the terrorist sarin attack on the Tokyo subway in 1995 by members of the Japanese cult Aum Shinrikyo. The attack resulted in 12 deaths and injuries to more than 5500 civilians [2,4]. OP nerve agents are likely to be a weapon of choice for many other terrorist organizations as well because they are relatively accessible or simple to produce, easy to transport, and can be delivered in mass quantities [5,6].

2. Nerve Agent Toxicity

2.1. Route of exposure

The various OP nerve agents differ in their water solubility and volatility, which influences an agent's route of exposure and potential as a chemical warfare agent. Most nerve agents exist as liquids and are readily absorbed through the skin and eyes; however, the G-agents (especially sarin) are highly volatile. Therefore, inhalation exposure also poses a threat. Unlike the G-agents, the V-agents have an oil-like consistency; therefore, the route of exposure for V-agents is considered to be primarily dermal. However, it should be noted that VX, which is the least volatile nerve agent, can vaporize in high temperatures or explosive conditions. In military use, V-agents would most likely be aerosolized into tiny droplets or vaporized for dispersion, thereby easily entering the body through the skin and mucous membranes or through inhalation [7,8]. Although ingestion of nerve agents is expected to be relatively rare compared to inhalation exposure or skin contact, they are readily absorbed from the GI tract and are highly toxic.

2.2. Acute toxicity of OP nerve agents

Neurotransmitters, such as acetylcholine (ACh), are endogenous chemicals that transmit signals from a neuron to a target cell across the chemical synapse, which allows the nervous system to connect to and control other systems of the body. The binding of ACh to its receptor sites on the postsynaptic membrane stimulates actions in the body such as breathing, digestion, and muscular contraction. The enzyme acetylcholinesterase (AChE) then inactivates ACh to terminate this synaptic transmission and allow the muscle, gland, or organ to relax.

It is well established that the acute toxicity of OP nerve agents results from the irreversible inhibition of AChE through the formation of a

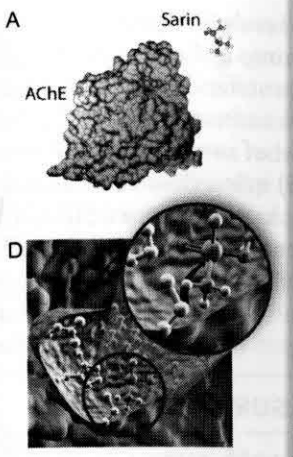


Figure 2 Interaction of sari
and AChE. (B) Entry of sari
sarin in the active site of A
AChE is a triad comprising
binding to the catalytic serin
ment to AChE, the fluoride i
molecule. (F) The active site
can then undergo dealkylati
irreversible enzyme inhibitio

covalent bond between t
ACh normally undergo
AChE is a triad compris
Nerve agents bind to the
and inhibiting cholineste
reactivated by a strong n
neurotoxicant from the
reactivation is depender
inhibited and the ther
AChE, a portion of the
from the bound molecu
kylation in a process ref
irreversible enzyme inhil
the aged OP–enzyme c
agents is variable; it can l
VX [10]. This AChE i
cholinergic synapses anc
nicotinic ACh receptors
system, including the ne

Material may be protected by copyright law (Title 17, U.S. Code)

piling, and use of chemical warfare
o prevent their use, OP nerve agents
ne of war as well as to the civilian
tack, such as the terrorist sarin attack
members of the Japanese cult Aum
deaths and injuries to more than
are likely to be a weapon of choice
as well because they are relatively
o transport, and can be delivered in

their water solubility and volatility,
xposure and potential as a chemical
as liquids and are readily absorbed
the G-agents (especially sarin) are
exposure also poses a threat. Unlike
like consistency; therefore, the route
to be primarily dermal. However, it
the least volatile nerve agent, can
losive conditions. In military use,
ized into tiny droplets or vaporized
g the body through the skin and
ation [7,8]. Although ingestion of
ively rare compared to inhalation
dily absorbed from the GI tract and

gents

e (ACh), are endogenous chemicals
o a target cell across the chemical
em to connect to and control other
ACh to its receptor sites on the
ons in the body such as breathing,
The enzyme acetylcholinesterase
inate this synaptic transmission and
lax.
toxicity of OP nerve agents results
ChE through the formation of a

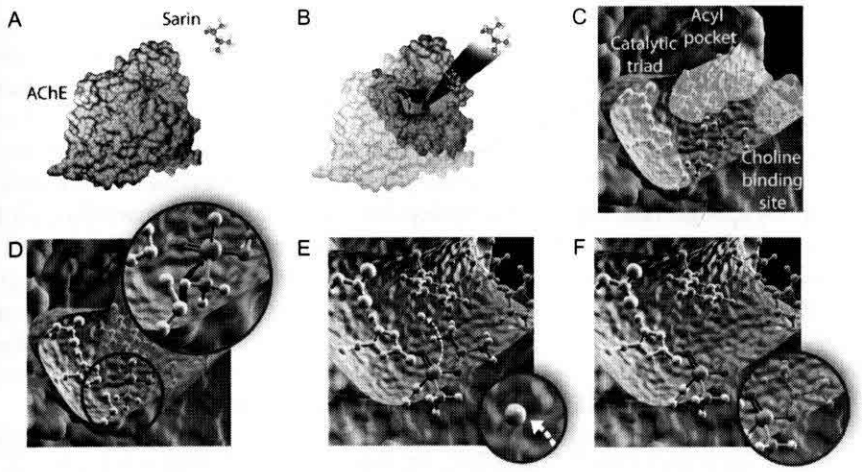


Figure 2 Interaction of sarin with acetylcholinesterase (AChE). (A) Structures of sarin and AChE. (B) Entry of sarin into the active site gorge of AChE. (C) Orientation of sarin in the active site of AChE relative to the catalytic triad. The catalytic center of AChE is a triad comprising serine-200, histidine-440, and glutamate-327. (D) Sarin binding to the catalytic serine, which prevents binding of ACh. (E) Following attachment to AChE, the fluoride ion of sarin (the leaving group) is cleaved from the bound molecule. (F) The active site serine is covalently adducted. The sarin bound to AChE can then undergo dealkylation in a process referred to as "aging," which results in irreversible enzyme inhibition. Illustration by Alexandre Katos.

covalent bond between the nerve agent and the site on the enzyme where ACh normally undergoes hydrolysis (Figure 2). The catalytic center of AChE is a triad comprising serine-200, histidine-440, and glutamate-327. Nerve agents bind to the catalytic serine, thereby preventing ACh binding and inhibiting cholinesterase (ChE) activity. The inhibited enzyme can be reactivated by a strong nucleophile, such as an oxime, which removes the neurotoxicant from the AChE molecule. However, the efficacy of AChE reactivation is dependent on the nerve agent with which the ChE was inhibited and the therapeutic oxime used. Following attachment to AChE, a portion of the nerve agent, called the leaving group, is cleaved from the bound molecule. OPs bound to AChE can then undergo dealkylation in a process referred to as "aging." This aged complex results in irreversible enzyme inhibition. Oximes have no effect and cannot reactivate the aged OP–enzyme conjugate [9]. The aging half-time for OP nerve agents is variable; it can be as short as 2 min for soman or as long as 48 h for VX [10]. This AChE inhibition causes a toxic accumulation of ACh at cholinergic synapses and results in the overstimulation of muscarinic and nicotinic ACh receptors (nAChRs) in the central and peripheral nervous system, including the neuromuscular junction [6,11,12].

Material may be protected by copyright law (Title 17, U.S. Code)

Acute exposure to an OP nerve agent initially results in symptoms such as myosis, tightening of the chest, difficulty breathing, and a general loss of bodily functions. As symptoms progress, the victim suffers from convulsive spasms and seizures, which can lead to death if left untreated [6,11–15]. In animals surviving an experimental convulsive dose of nerve agent, spiking activity appears in the electroencephalogram (EEG) in less than 10 min, and it rapidly progresses into status epilepticus that lasts for hours [16,17]. Although the intensity of seizures decreases over time, the EEG is still abnormal days after the intoxication [18].

3. Current Medical Countermeasures to Nerve Agent Toxicity

OP nerve agents can be fatal at low concentrations, but even a nonlethal dose can cause permanent neurological damage if the person does not receive proper medical attention immediately. Based on the previous scientific findings discussed in the section above, current medical countermeasures to nerve agent intoxication include an anticholinergic (e.g., atropine) that blocks excess ACh at muscarinic receptors to alleviate parasympathetic overstimulation, an oxime (e.g., 2-pyridine aldoxime methylchloride [2-PAM] or 1-2-hydroxylminomethyl-1-pyridino-3-(4-carbamoyl-1-pyridino-2-oxapropane dichloride) [HI-6]) to reactivate inhibited AChE molecules, and an anticonvulsant such as diazepam [11–13,19]. These therapeutics increase survival if administered within a short period of time following exposure, but they may not fully prevent neurological damage or functional impairment [2,6,11,15,20–22]. This is of great concern because although these countermeasures are readily available to soldiers in a combat setting, they are not accessible to the general public in case of a terrorist attack. Rapidly terminating nerve agent-induced seizures is critical because their duration and intensity have been directly linked to brain damage following exposure [19,23–25]. Studies have shown signs of neuropathology present within 20 min of seizure onset and have demonstrated the increased difficulty in terminating seizures that have lasted beyond 40 min [15,23]. These findings are important considering that the anticipated response time to treat civilian casualties exposed to nerve agent is estimated to be at least 30 min [15], and many will have already initiated seizures by the time medical personnel arrive.

Survivors of nerve agent poisoning can experience long-term neurological and behavioral outcomes months or years following exposure [2]. Previous findings by Scremin *et al.* [26] revealed that sarin-exposed rats showed behavioral abnormalities up to 16 weeks post-exposure. To date, most of our understanding on this issue in humans comes from studies performed on

survivors of the Tokyo sul
reported on the long-term
means of an improvised ex
in 2004. Testing performe
victim suffered from redu
attention, and difficulty i
long-term neurologic sec
Therefore, the molecular
agent-induced neurodege
treatments that would be
seizures and secondary res

4. Global Mol
Enable the
Mechanisms

To date, most scie
involved classical toxicol
histopathology, and ACh
exposure. While these r
exposure, they are not as
toxicity or providing insig
help determine the molec
have recently begun usin
effects and physiological n
AChE inhibition. In the
large sets of biological mol
genomics (focused on th
referred to as "transcripto
(focused on large sets of p
on large sets of small mole
ogies offer a global scree
subsequent bioinformatic
ways and molecular funct

4.1. Genomics

Before genomics, molec
real-time polymerase cha
tify the expression level
"big picture" of gene fun
as the introduction of chi

Material may be protected by copyright law (Title 17, U.S. Code)

nt initially results in symptoms such
ulty breathing, and a general loss of
the victim suffers from convulsive
death if left untreated [6,11–15]. In
ulsive dose of nerve agent, spiking
ram (EEG) in less than 10 min, and
ticus that lasts for hours [16,17].
reases over time, the EEG is still
].

TERMEASURES
Y

low concentrations, but even a
eurological damage if the person
ntion immediately. Based on the
the section above, current medical
cation include an anticholinergic
at muscarinic receptors to alleviate
xime (e.g., 2-pyridine aldoxime
oxylminomethyl-1-pyridino-3-(4-
ichloride) [HI-6]) to reactivate
anticonvulsant such as diazepam
survival if administered within a
e, but they may not fully prevent
rment [2,6,11,15,20–22]. This is of
untermeasures are readily available
not accessible to the general public
terminating nerve agent-induced
n and intensity have been directly
re [19,23–25]. Studies have shown
20 min of seizure onset and have
erminating seizures that have lasted
are important considering that the
casualties exposed to nerve agent is
d many will have already initiated
rrive.
n experience long-term neurologi-
ears following exposure [2]. Previ-
led that sarin-exposed rats showed
post-exposure. To date, most of our
comes from studies performed on

survivors of the Tokyo subway attack. More recently, Loh and colleagues [5] reported on the long-term cognitive sequelae of a soldier exposed to sarin by means of an improvised explosive device (IED) while he was deployed to Iraq in 2004. Testing performed 10 months following exposure revealed that the victim suffered from reduced information processing speed, poor focused attention, and difficulty in motor coordination. Despite these studies, the long-term neurologic sequelae of nerve agent exposure are still unclear. Therefore, the molecular effects and biological pathways involved in nerve agent-induced neurodegeneration need to be examined to determine drug treatments that would be effective when administered after the onset of seizures and secondary responses that lead to brain injury.

4. Global Molecular Screening Approaches Enable the Identification of Molecular Mechanisms of Toxicant Exposure

To date, most scientific investigations of nerve agent toxicity have involved classical toxicology where physical symptoms of exposure, tissue histopathology, and AChE activity are used to indicate adverse effects of exposure. While these measurements are useful indicators of acute OP exposure, they are not as effective in studying the molecular mechanisms of toxicity or providing insight into the long-term effects of OP exposure. To help determine the molecular mechanisms of nerve agent toxicity, scientists have recently begun using "omics" technologies to assess the toxicological effects and physiological mechanisms of OP nerve agents, expanding beyond AChE inhibition. In the life sciences, the suffix *omics* refers to the study of large sets of biological molecules [27]. This field of molecular biology includes genomics (focused on the genome sequence or genomic expression, also referred to as "transcriptomics" or "gene expression profiling"), proteomics (focused on large sets of proteins, the proteome), and metabolomics (focused on large sets of small molecules, the metabolome). These multiplex technologies offer a global screening approach for OP nerve agent toxicity, and subsequent bioinformatic analyses enable the identification of biological pathways and molecular functions significantly affected by nerve agent exposure.

4.1. Genomics

Before genomics, molecular biology techniques (e.g., Northern blot or real-time polymerase chain reaction [PCR]) were used to detect and quantify the expression level of individual genes, making it difficult to see the "big picture" of gene function in a cell. Technological developments, such as the introduction of chip-based assays (e.g., DNA microarrays), have had a

Material may be protected by copyright law (Title 17, U.S. Code)

major impact on the resolution and throughput of omic-based studies. Since their development in the 1990s, DNA microarrays have had an important influence on the fields of molecular biology and toxicology. These arrays are commonly used to simultaneously measure the mRNA (mRNA) levels of thousands of genes in a cell and are capable of detecting even subtle changes in gene expression. DNA arrays monitor the expression of a gene by measuring its level of hybridization to a target sequence localized to a specific region on an array. In brief, RNA is extracted from a biological sample of interest and used as a template to synthesize labeled nucleic acid probe. Labeled probe is then hybridized to the array that contains thousands of gene targets and binds to complementary target sequences on the array. Quantitative imaging coupled with clone database information allows measurement of the amount of labeled probe that hybridized to each target sequence, resulting in the identification and relative quantification of the genes expressed in the biological sample [28,29]. Given that this technique provides a quantitative overview of the mRNA transcripts present in a biological sample by detecting the genes that are up- or down-regulated at any given time and in any physiological situation, it can be used to determine which genes are differentially expressed as a result of nerve agent exposure and aid in identifying mechanisms of toxicity. Using a global approach to analyze thousands of genes in a single experiment, it is easier to assay the overall molecular response of a cell, tissue, or organ and then identify specific genes and/or groups of genes altered following a chemical exposure. Moreover, these global molecular techniques are capable of identifying differentially expressed molecules between samples without any prior knowledge of their functions. Thus, scientists can detect potentially interesting genes and molecular mechanisms that were not previously suspected to be important for biological processes and can generate new biological hypotheses [30].

4.2. Proteomics

Although gene expression profiling provides many advantages for studying the molecular mechanisms of nerve agent toxicity, such as being able to use one biological sample to measure the expression of thousands of genes simultaneously, DNA microarrays can only monitor cellular responses at the mRNA level and provide little information regarding the functional state of the proteins they encode. Even though all proteins are based on mRNA precursors, post-translational modifications and environmental interactions make it impossible to predict abundance of specific proteins based on gene expression analysis alone. Previous studies have shown that mRNA is not always translated into protein, and the amount of protein produced depends on the physiological state of the cell. Further, a single gene can give rise to a number of functionally different proteins through various

molecular processes suc
carbohydrate residues to
to serine, threonine, or
data need to be comple
mine the molecular me

Many proteomic tec
abundance and some as
involve the physical se
dodecyl sulfate polyac
dimensional SDS-PAG
ion-exchange chromat
chromatography, or hi
separated, proteins of int
detection used in protei
capacity to detect only c
valuable for a detailed
provide information or
system, unless a large r
Combining a protein s
the potential to overcor
analysis techniques. The
ments in protein identifi
MS-based analyses of co
gel-based methods, suc
low throughput with
addition of stable isotc
identification. A shotgu
tags for relative and abs
tides for the simultaneo
or more samples in a sing
varying mass are used t
biological samples. The
separated by two-dimen
etry (MS/MS) to identi
been successfully used t
[33]. Phosphorylation e
chromatography (IMAC
for signaling phosphopr
phosphorylated at low s
phorylation status of the
and IMAC techniques
pathways by probing m
approach would permit
nerve agent exposure an

Material may be protected by copyright law (Title 17, U.S. Code)

oughput of omic-based studies. Since
A microarrays have had an important
ology and toxicology. These arrays are
asure the mRNA (mRNA) levels of
able of detecting even subtle changes
onitor the expression of a gene by
to a target sequence localized to a
RNA is extracted from a biological
ate to synthesize labeled nucleic acid
d to the array that contains thousands
entary target sequences on the array.
one database information allows mea-
robe that hybridized to each target
on and relative quantification of the
le [28,29]. Given that this technique
the mRNA transcripts present in a
nes that are up- or down-regulated
ological situation, it can be used to
ially expressed as a result of nerve
echanisms of toxicity. Using a global
s in a single experiment, it is easier to
of a cell, tissue, or organ and then
of genes altered following a chemical
olecular techniques are capable of
olecules between samples without
s. Thus, scientists can detect poten-
nechanisms that were not previously
ical processes and can generate new

ovides many advantages for studying
ent toxicity, such as being able to use
e expression of thousands of genes
only monitor cellular responses at
nformation regarding the functional
en though all proteins are based on
odifications and environmental inter-
undance of specific proteins based on
s studies have shown that mRNA is
d the amount of protein produced
the cell. Further, a single gene can
different proteins through various

molecular processes such as alternative splicing of pre-mRNAs, attachment of carbohydrate residues to form glycoproteins, or addition of phosphate groups to serine, threonine, or tyrosine amino acids in the protein. Thus, genomic data need to be complemented with protein expression data to truly determine the molecular mechanisms of toxicity of nerve agents.

Many proteomic technologies are currently available to quantify protein abundance and some aspects of the activation state. Proteomic studies usually involve the physical separation of proteins by techniques such as sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), two-dimensional SDS-PAGE (2D-PAGE), or column chromatography (e.g., ion-exchange chromatography, gel filtration chromatography, affinity chromatography, or high-pressure liquid chromatography [HPLC]). Once separated, proteins of interest can then be detected. The traditional methods of detection used in protein chemistry, such as Western blot analysis, have the capacity to detect only one protein at a time. Although this approach remains valuable for a detailed understanding of a protein of interest, it does not provide information on the interactions between numerous proteins in a system, unless a large number of consecutive experiments are performed. Combining a protein separation method with mass spectrometry (MS) has the potential to overcome the limitations encountered in traditional protein analysis techniques. The rapid evolution in MS has led to significant improvements in protein identification. Combining liquid chromatography (LC) with MS-based analyses of complex mixtures overcomes many of the drawbacks to gel-based methods, such as limited dynamic range and sensitivity, as well as low throughput with respect to protein identification [31]. Further, the addition of stable isotopes to the proteome allows simultaneous protein identification. A shotgun proteomic technique, such as iTRAQ (isobaric tags for relative and absolute quantitation), involves isotopic labeling of peptides for the simultaneous identification and quantification of proteins in two or more samples in a single experiment [32]. With this method, isobaric tags of varying mass are used to label the primary amines of peptides from different biological samples. The labeled peptides from each sample are then mixed, separated by two-dimensional LC, and analyzed using tandem mass spectrometry (MS/MS) to identify global changes in the proteome. This approach has been successfully used to study global protein expression in neuronal systems [33]. Phosphorylation enrichment schemes, such as immobilized metal affinity chromatography (IMAC) [34], can also be used prior to MS analysis to enrich for signaling phosphoproteins, which are usually of low abundance and/or are phosphorylated at low stoichiometry. Identifying changes in the global phosphorylation status of the proteome upon nerve agent exposure using iTRAQ and IMAC techniques can be used to characterize proteome-wide signaling pathways by probing many signaling phosphoproteins simultaneously. This approach would permit the identification of molecular pathways perturbed by nerve agent exposure and elucidate mechanisms of toxicity.

Material may be protected by copyright law (Title 17, U.S. Code)

The newest approach to analyzing the proteome involves the use of protein microarrays, which are based on the same concept as DNA microarrays discussed above. The most common protein microarrays are antibody arrays used to profile a complex mixture of proteins and measure protein expression levels in the mixture [35]. Although simple in principle, protein microarrays are more difficult to work with than DNA microarrays because proteins vary in their chemistry (e.g., hydrophobic vs. hydrophilic), and they bind to each other by several types of noncovalent interactions. Fragments of DNA, however, vary only in their nucleotide sequence and bind to their partners by simple Watson–Crick base pairing. Despite these challenges, this field of proteomic analysis is expected to make rapid progress and to move toward standardized protocols as with transcriptomics.

4.3. Metabolomics

The newest field of global molecular profiling is metabolomics. Metabolic phenotypes are the by-products that result from the interaction between genetic, environmental, and other factors [27]. In contrast to the genome and proteome, the metabolome consists of small molecules that are also known as metabolites. As their name suggests, metabolites are involved in the energy transmission, or metabolism, in cells. Metabolic profiling is performed on easily collected biological samples such as urine, saliva, or plasma. The metabolome consists of a wide range of chemical structures, and like the genome and proteome, it is highly variable and time-dependent following toxicant exposure. The techniques currently used in metabolomics give researchers the ability to make an unbiased assessment of biochemical status at the cellular, tissue, or organism level. MS and nuclear magnetic resonance (NMR) are the primary technologies used for metabolomic studies [36]. Both technologies have made recent advances in sensitivity and data manipulation and storage. MS is the most common technique due to its high accuracy and sensitivity. The main advantage of NMR is that it does not require any upfront chromatographic separation, and it does not destroy the samples during analysis. NMR has the ability to measure molecular compartmentalization and dynamic biochemical changes in real time. An important challenge of metabolomics is to acquire qualitative and quantitative information concerning the metabolites that occur under normal circumstances and detect perturbations in the complement of metabolites as a result of changes in environmental factors [27].

4.4. Bioinformatic tools

Although these global molecular techniques are powerful tools to explore the expression levels of thousands of genes, proteins, or metabolites after toxicant exposure, a major challenge lies in the interpretation of the massive

amounts of data collected i
microarray technology in
statistical analyses and data
tools are being applied in

In the past few years,
software programs for com
These bioinformatic tools
datasets and have allowed s
molecular mechanisms in
during nerve agent poison
of molecular techniques, it
will again surpass our capa
scientists must continually
methods to interpret these

5. Global Mole Three-Phase

The neuropathology
cally thought to result fro
damage occurs in numero
hippocampus, piriform co
In a three-phase model p
initiation is a cholinergic p
to ~5 min after seizure on
soman immediately inhibi
10 min and a large increa
initiation [41,42]. If seizur
occurs 5–40 min post-exp
perturbed. During this ph
such as glutamate, increase
after the onset of soman-in
likely due to increased hyd
phase of the model, which
non, starting ~40 min aft
activity is maintained from
and that the glutamatergic
aspartate (NMDA) calciu
intracellular calcium [11,1
that cause neuronal death.
the ultimate cause of neur
can hyperactivate enzymes

Material may be protected by copyright law (Title 17, U.S. Code)

g the proteome involves the use of
on the same concept as DNA micro-
non protein microarrays are antibody
ure of proteins and measure protein
Although simple in principle, protein
with than DNA microarrays because
hydrophobic vs. hydrophilic), and
es of noncovalent interactions. Frag-
their nucleotide sequence and bind
-Crick base pairing. Despite these
ysis is expected to make rapid prog-
protocols as with transcriptomics.

profiling is metabolomics. Metabolic
result from the interaction between
tors [27]. In contrast to the genome
sts of small molecules that are also
suggests, metabolites are involved in
sm, in cells. Metabolic profiling is
cal samples such as urine, saliva, or
wide range of chemical structures,
highly variable and time-dependent
niques currently used in metabolo-
ake an unbiased assessment of bio-
or organism level. MS and nuclear
mary technologies used for metabo-
have made recent advances in sensi-
orage. MS is the most common
sensitivity. The main advantage of
ront chromatographic separation, and
g analysis. NMR has the ability to
n and dynamic biochemical changes
metabolomics is to acquire qualitative
ng the metabolites that occur under
bations in the complement of meta-
mental factors [27].

iques are powerful tools to explore
genes, proteins, or metabolites after
s in the interpretation of the massive

amounts of data collected in each scientific study. The rapid advancement of microarray technology increased the need for integration of higher-order statistical analyses and data management (e.g., [37]), and these same analysis tools are being applied in the field of proteomics and metabolomics.

In the past few years, there has been rapid advanced development of software programs for comprehensive pathway analysis and literature mining. These bioinformatic tools have enabled the interpretation of large, complex datasets and have allowed scientists to better identify biological pathways and molecular mechanisms involved in toxicant exposure, such as those seen during nerve agent poisoning. However, with the continuing advancement of molecular techniques, it is likely that the methods used for collecting data will again surpass our capacity to adequately analyze the results. Therefore, scientists must continually develop more advanced bioinformatic tools and methods to interpret these large datasets.

5. Global Molecular Techniques Support the Three-Phase Model of Nerve Agent Toxicity

The neuropathology resulting from OP nerve agent exposure is typically thought to result from sustained seizure activity. Seizure-related brain damage occurs in numerous sensitive areas in the brain such as the amygdala, hippocampus, piriform cortex, septum, and thalamus [6,15,23,25,38–40]. In a three-phase model proposed by McDonough and Shih [11], seizure initiation is a cholinergic phenomenon that lasts from the time of exposure to ~5 min after seizure onset. It has been reported that a convulsant dose of soman immediately inhibits brain ChE with maximum inhibition within 10 min and a large increase in ACh concentration at the time of seizure initiation [41,42]. If seizures are not stopped immediately, a transition phase occurs 5–40 min post-exposure in which other neurotransmitter systems are perturbed. During this phase, the level of excitatory amino acids (EAAs), such as glutamate, increases and potentiates seizure activity [11,43]. Shortly after the onset of soman-induced seizures, choline (Ch) levels increase [41], likely due to increased hydrolysis of phospholipids [44]. This leads to the last phase of the model, which is predominantly a non-cholinergic phenomenon, starting ~40 min after seizure onset. It is postulated that the seizure activity is maintained from this point on by excessive glutamate stimulation, and that the glutamatergic hyperactivity causes an opening of *N*-methyl-D-aspartate (NMDA) calcium channels, leading to subsequent increases in intracellular calcium [11,13,45], which in turn initiates signaling cascades that cause neuronal death. It is proposed that this excess influx of calcium is the ultimate cause of neuropathology following nerve agent exposure as it can hyperactivate enzymes such as lipases, proteases, endonucleases, kinases,

Material may be protected by copyright law (Title 17, U.S. Code)

or phosphatases that can cause damage to cell membranes and the cytoskeleton, as well as to organelle structure and function [18,45].

A large amount of data obtained from scientific studies utilizing global molecular techniques support the temporal model proposed by McDonough and Shih [11] that links nerve agent-induced seizures to resulting neuropathology. Studies using microarray analysis have shown that many genes and signaling pathways are altered within the first 15 min after acute sarin exposure [39,40,46]. Among these significantly altered genes are those involved in cholinergic signaling, catecholaminergic signaling (which modulates seizure susceptibility in animal models of epilepsy [47]), γ-aminobutyric acid (GABA)ergic signaling, glutamate and aspartate signaling, calcium channels and binding proteins, neurotransmission and neurotransmitter transporters, neuropeptides, and ligand-gated ion channels that open and close in response to the binding of a neurotransmitter (e.g., nAChR, GABA receptor, and NMDA receptor [46]). At 2-h post-exposure, Damodaran and colleagues [48] continued to observe sarin-induced alterations in the mRNA expression of neurotransmitter transporters (such as synaptojanin I, syntaxin 6, and SVO), signaling receptors (such as the GABA-A receptor rho-2 subunit and AMPA2), and ligand-gated ion channels. The transcriptional response at these early time points following acute sarin exposure clearly relates to the three phases of nerve agent toxicity and supports the model proposed by McDonough and Shih [11].

Spradling and colleagues also used oligonucleotide microarrays to study the changes in gene expression profiles after a $1\times$ LD_{50} sarin exposure in a rat model over a 24-h time period [39,40]. Consistent with the findings of Damodaran *et al.* [46,48], the findings of Spradling *et al.* at 15 min after seizure onset support the transition phase of the model. For example, D-glutamine and D-glutamate metabolism and glutamate receptor signaling were two of the canonical pathways significantly altered immediately after seizure onset. Previous studies have shown an increase in choline, a precursor for ACh, 15–30 min after nerve agent exposure [41] due to increased hydrolysis of phospholipids [44], which supports the presence of phospholipid degradation among the significant pathways at this early time point in the work by Spradling *et al.* The most significantly altered pathway at 15 min was inositol metabolism. Inositol works closely with Ch as a primary component of cell membranes. It is necessary for normal nerve and brain function as it is required for proper action of several neurotransmitters, such as ACh and serotonin. Studies have shown that membrane phosphoinositide (PI) is hydrolyzed following the activation of neurotransmitter receptors, such as NMDA, to yield inositol 1,4,5-triphosphate (IP_3), a second messenger that transmits signals from the receptor into the cell by releasing calcium from non-mitochondrial intracellular stores [11,13,45].

In addition to these earlier time points, Damodaran and colleagues [46] also examined the transcriptional response at 3 months following sarin

exposure. The authors r
dependent kinase II (C
calcium transporter m
changes persist for a lon
induced pathology [46].
which is integrally invo
learning and memory,
receptors, resulting in mi
followed by caspase acti
regions [46]. Uncoverin
is complicated due to th
programmed cell death [

6. Global Mo Evidence fo Nerve Agen Effects of

Since the developn
Shih [11], studies using
presence of non-choline
phase response to nerve
effects leading to nerve
conducted one of the firs
identify genes altered b
employed microarray an
low-level (0.1, 0.2, and
altered expression profile
and cortex following exp
after the final exposure. I
Blanton and colleagues
ters of genes that were si
neuronal genes encodin
motors as well as genes a
upregulated in the hipp
similar functional catego
the authors identified
following VX exposure,
pathway from the datase

Since the study by E
global molecular techni
responses of sensitive bra

Material may be protected by copyright law (Title 17, U.S. Code)

o cell membranes and the cytoskel-
d function [18,45].
from scientific studies utilizing
he temporal model proposed by
nerve agent-induced seizures to
g microarray analysis have shown
are altered within the first 15 min
Among these significantly altered
ignaling, catecholaminergic signal-
ility in animal models of epilepsy
signaling, glutamate and aspartate
g proteins, neurotransmission and
ides, and ligand-gated ion channels
inding of a neurotransmitter (e.g.,
receptor [46]). At 2-h post-expo-
ontinued to observe sarin-induced
neurotransmitter transporters (such
), signaling receptors (such as the
AMPA2), and ligand-gated ion
t these early time points following
three phases of nerve agent toxicity
Donough and Shih [11].
igonucleotide microarrays to study
fter a 1× LD_{50} sarin exposure in a
0]. Consistent with the findings of
of Spradling *et al.* at 15 min after
ase of the model. For example,
n and glutamate receptor signaling
nificantly altered immediately after
n an increase in choline, a precur-
nt exposure [41] due to increased
supports the presence of phospho-
athways at this early time point in
t significantly altered pathway at
works closely with Ch as a primary
essary for normal nerve and brain
of several neurotransmitters, such
wn that membrane phosphoinosi-
ivation of neurotransmitter recep-
1,4,5-triphosphate (IP_3), a second
receptor into the cell by releasing
llular stores [11,13,45].
s, Damodaran and colleagues [46]
nse at 3 months following sarin

exposure. The authors noted persistent alteration of calcium/calmodulin-dependent kinase II (CamKII) from 2 h to 3 months (along with other calcium transporter molecules), which indicates that calcium-induced changes persist for a long time and likely play an important role in sarin-induced pathology [46]. Day and Greenfield [49] suggested that CamKII, which is integrally involved with glutamate receptors in the processes of learning and memory, is activated by calcium influx through NMDA receptors, resulting in mitochondrial dysfunction and free radical formation, followed by caspase activation and apoptotic cell death of sensitive brain regions [46]. Uncovering the roles of calcium during nerve agent poisoning is complicated due to the role calcium plays in both neuronal survival and programmed cell death [50].

6. Global Molecular Techniques Provide Evidence for Non-AChE Mechanisms of OP Nerve Agent Toxicity and Reveal Secondary Effects of Exposure

Since the development of the three-phase model by McDonough and Shih [11], studies using global molecular techniques have also indicated the presence of non-cholinergic and non-glutamatergic systems during the late phase response to nerve agents, providing critical insight into the secondary effects leading to nerve agent-induced neuropathology. Blanton *et al.* [51] conducted one of the first high-density gene expression profiling analyses to identify genes altered by nerve agent exposure. In this study, the authors employed microarray analysis to analyze gene responses following a 2-week low-level (0.1, 0.2, and 0.4 LD_{50}) exposure to VX. Their results revealed altered expression profiles of non-AChE targets in the mouse hippocampus and cortex following exposure, which returned to normal levels by 2 weeks after the final exposure. In this early genomic study of nerve agent exposure, Blanton and colleagues used self-organizing maps (SOMs) to identify clusters of genes that were similarly affected by VX poisoning. They identified neuronal genes encoding receptors, vesicle-related proteins, and molecular motors as well as genes associated with neurogenesis/maintenance as being upregulated in the hippocampus following exposure. They also identified similar functional categories of genes being altered in the cortex. Although the authors identified numerous genes with altered expression patterns following VX exposure, they were unable to identify a complete alternate pathway from the dataset.

Since the study by Blanton *et al.* [51], scientists have continued using global molecular techniques to understand in greater detail the molecular responses of sensitive brain regions to nerve agent exposure. The correlation

Material may be protected by copyright law (Title 17, U.S. Code)

between neuronal activity and the expression of specific genes has led to an increase of scientific data related to the long-lasting functional changes that underlie nerve agent toxicity [48]. Gene expression profiling has been successfully used to investigate the mechanisms of toxicity and resulting effects of sarin [39,40,46,48,52] and soman [53]. In 2006, Damodaran and colleagues published two papers detailing sarin-induced gene expression changes in male Sprague–Dawley rat brains following a $0.5\times$ LD_{50} exposure at 15 min [46] and 2 h [48] and a $1\times$ LD_{50} exposure at a later time point of 3 months. In their studies, the animals were challenged with the appropriate dose of sarin (im, diluted in saline), and the animals were anesthetized with 100 μg/kg ketamine/xylazine prior to brain dissection. In the study by Dillman *et al.* [53], male Sprague–Dawley rats were pretreated with 125 mg/kg of the oxime HI-6 (ip) 30 min prior to exposure of 180 μg/kg soman (sc, diluted with 0.9% sodium chloride). One minute after soman challenge, the animals were treated with atropine methyl nitrate (2 mg/kg, im). The hippocampi were immediately collected after deep anesthesia using sodium pentobarbital (65 mg/kg, ip) at 1, 3, 6, 12, 24, 48, 72, 96, and 168 h after the onset of convulsions. The microarray studies by Spradling and colleagues in 2010 [39,40] also involved the use of male Sprague–Dawley rats. The animals were challenged with $1\times$ LD_{50} sarin (sc, diluted in saline). One minute after seizure onset, the animals were treated with atropine sulfate (2 mg/kg) and the oxime 2-PAM (25 mg/kg), both administered in a single injection (im). Thirty minutes later, the animals were given the anticonvulsant diazepam (10 mg/kg, sc). The amygdala, hippocampus, piriform cortex, septum, and thalamus were immediately collected at 15 min, 1, 3, 6, and 24 h after seizure onset following decapitation. These studies helped uncover the secondary molecular effects of intracellular calcium overload that are expected to produce important cerebral biochemical and metabolic disruption [18].

6.1. Oxidative stress

Previous data have shown that the hyperactivation of enzymes resulting from intracellular calcium overload during nerve agent poisoning promotes oxidative stress [54]. Results have implicated exposure to OP nerve agents in the generation of free radicals (e.g., hydrogen peroxide [H_2O_2], superoxide [O_2^-], nitric oxide [NO], and peroxynitrite [$ONOO^-$]) and the alteration of the antioxidative scavenging system. The accumulation of reactive oxygen species during an oxidative stress response induces various defense mechanisms or programmed cell death. Damodaran and colleagues [46] noted an induction of nitric oxide synthase (NOS)-2 mRNA expression following sarin exposure, which supported their previous finding of nitrotyrosine production in sarin-exposed rats [55]. Further, Damodaran *et al.* [46] saw a reduction in mRNA expression level for glutathione

S-transferase A2 (GSTA2
nism for minimizing oxi
that this reduction in G
GABA populations due
dopamine receptor type

The occurrence of ox
linked to numerous dow
targets of oxidative dam
mitochondrial DNA. O
those involved in modu
target macromolecules, a
cells [46]. One of the cor
which has previously bee
was further supported by
sion [46]. One mechanis
toxicity is through the act
production of HSPs has
[39,40,48,59]. These pr
during nerve agent pois
protein folding, and regu

6.2. Mitochondrial d

Mitochondria are respons
oxidative phosphorylatio
cellular processes that re
suggesting that mitochon
seizures [60]; therefore, i
following nerve agent ex

Previous studies have
for intracellular calcium
results from mitochondri
enge the excess calcium p
mitochondria may be su
exceed a physiological th

Another important tr
reactive oxygen species,
drial permeability transiti
These oxygen radicals a
chain is inhibited [61,62
species are produced, the
(e.g., glutathione peroxi
in the oxidative damage
natively, oxygen radic

Material may be protected by copyright law (Title 17, U.S. Code)

pression of specific genes has led to an
ıe long-lasting functional changes that
Gene expression profiling has been
mechanisms of toxicity and resulting
soman [53]. In 2006, Damodaran and
ailing sarin-induced gene expression
brains following a 0.5× LD_{50} expo-
1× LD_{50} exposure at a later time point
nals were challenged with the appro-
ne), and the animals were anesthetized
ior to brain dissection. In the study by
-Dawley rats were pretreated with
30 min prior to exposure of 180 μg/
m chloride). One minute after soman
ith atropine methyl nitrate (2 mg/kg,
ately collected after deep anesthesia
kg, ip) at 1, 3, 6, 12, 24, 48, 72, 96,
ns. The microarray studies by Sprad-
so involved the use of male Sprague–
ged with 1× LD_{50} sarin (sc, diluted in
nset, the animals were treated with
ime 2-PAM (25 mg/kg), both admi-
hirty minutes later, the animals were
0 mg/kg, sc). The amygdala, hippo-
thalamus were immediately collected
e onset following decapitation. These
ry molecular effects of intracellular
roduce important cerebral biochemical

nyperactivation of enzymes resulting
ring nerve agent poisoning promotes
plicated exposure to OP nerve agents
, hydrogen peroxide [H_2O_2], super-
d peroxynitrite [$ONOO^-$]) and the
nging system. The accumulation of
dative stress response induces various
cell death. Damodaran and colleagues
le synthase (NOS)-2 mRNA expres-
supported their previous finding of
posed rats [55]. Further, Damodaran
NA expression level for glutathione

S-transferase A2 (GSTA2), which serves as an important protective mechanism for minimizing oxidative damage in the cell. The authors speculate that this reduction in GSTA2 may potentiate damage to dopamine and GABA populations due to an alteration in the mRNA expression of dopamine receptor type 4 and GABA receptor, respectively.

The occurrence of oxidative stress during nerve agent poisoning can be linked to numerous downstream molecular effects. The macromolecular targets of oxidative damage include lipids, proteins, and both nuclear and mitochondrial DNA. Oxidative stress affects numerous genes, including those involved in modulating the production of free radicals, repairing target macromolecules, and specifying the orderly replacement of effector cells [46]. One of the consequences of oxidative stress is lipid peroxidation, which has previously been shown in the CNS of sarin-exposed rats [56] and was further supported by the observed alteration in lipophilin gene expression [46]. One mechanism in which the cells suppress oxidative-induced toxicity is through the activation of heat shock proteins (HSPs) [57,58]. The production of HSPs has been observed following nerve agent exposure [39,40,48,59]. These proteins are thought to provide cellular protection during nerve agent poisoning through their roles in protein trafficking, protein folding, and regulation of cell death pathways.

6.2. Mitochondrial dysfunction

Mitochondria are responsible for producing energy through the coupling of oxidative phosphorylation to respiration in order to provide ATP for most cellular processes that require energy. There is a large amount of evidence suggesting that mitochondrial dysfunction in the CNS is linked to epileptic seizures [60]; therefore, it may play a role in seizure-related brain damage following nerve agent exposure.

Previous studies have shown that neuronal mitochondria are important for intracellular calcium sequestration, so a major form of damage likely results from mitochondrial swelling as the mitochondria take up and scavenge the excess calcium produced during nerve agent poisoning. Therefore, mitochondria may be subjected to stress and even collapse if calcium levels exceed a physiological threshold [50].

Another important trigger for mitochondrial stress is the production of reactive oxygen species, which have been suggested to activate mitochondrial permeability transition pores and release mitochondrial cytochrome *c*. These oxygen radicals are generated when the mitochondrial respiratory chain is inhibited [61,62]. When excess amounts of these reactive oxygen species are produced, they can overload the endogenous protective enzymes (e.g., glutathione peroxidase, superoxide dismutase, and catalase) and result in the oxidative damage of proteins, phospholipids, and DNA [60]. Alternatively, oxygen radicals themselves can inhibit the mitochondrial

Material may be protected by copyright law (Title 17, U.S. Code)

respiratory chain [63] to create a very toxic and vicious cycle. This cyclic mechanism is thought to cause the progressive respiratory chain impairment in the CA1 and CA3 hippocampal subfields of pilocarpine-treated chronic epileptic rats [60,64].

In addition to the alteration of neuronal calcium homeostasis, mitochondrial dysfunction likely results in decreased intracellular ATP levels, which could increase neuronal excitability. This is supported by previous genomic studies indicating the significant alteration of cyclic nucleotide signaling and purinergic signaling within 15 min of sarin exposure [39,40,46]. Damodaran and colleagues [48] also noted the significant alteration of four ATPases and four ATP-based transporters at 2 h after sarin exposure. The data obtained in these studies support earlier findings showing a large increase in blood glucose concentration in the first hours of soman-induced seizures [65] and increased glucose utilization in nerve agent-damaged brain areas [66].

Status epilepticus-induced neuronal injury by the anticholinesterase diisopropylfluorophosphate (DFP) or the carbamate carbofuran has been linked to excitotoxicity, energy impairment, and oxidative stress [67]. The authors found an inverse relationship between an NO increase and a decrease in high-energy phosphates in the CNS of rats exposed to these AChE inhibitors. They attributed this finding to NO-induced impairment of mitochondrial respiration leading to depletion of energy metabolites. This was supported later by the findings of Damodaran *et al.* [46], who noted alterations in the transcription levels of brain Acyl-CoA synthetase and leptin along with a decrease in the mRNA levels of key mitochondria-associated proteins such as B-cell lymphoma (BCL)-2-associated X protein (i.e., BAX) and BCL-2-related ovarian killer protein (BOK). The authors suggest that OP chemicals may alter cytochrome P-450 and generate oxidants in their metabolic process that would subsequently oxidize a wide range of endogenous chemicals.

6.3. Apoptosis

In addition to supplying ATP for cellular functions, mitochondria also regulate apoptosis by controlling the release of mitochondrial proteins such as cytochrome *c* and other apoptotic factors. Members of the BCL-2 family are involved in the regulation of apoptosis. Some of the BCL-2 family members (including BCL-2, BCL-extra large [BCL-XL], and BCL-2-like protein 2 [BCL-W]) are considered to be anti-apoptotic, whereas other members (including BCL-2-associated death promoter [BAD], BAX, and BCL-2 homologous antagonist/killer [BAK]) induce apoptosis.

Although the neurotoxic mechanism of nerve agent poisoning has yet to be clearly defined, much of the scientific data suggest that it involves

Material may be protected by copyright law (Title 17, U.S. Code)

toxic and vicious cycle. This cyclic
gressive respiratory chain impairment
ofields of pilocarpine-treated chronic

onal calcium homeostasis, mitochon-
eased intracellular ATP levels, which
his is supported by previous genomic
ion of cyclic nucleotide signaling and
f sarin exposure [39,40,46]. Damo-
d the significant alteration of four
rters at 2 h after sarin exposure. The
rt earlier findings showing a large
n in the first hours of soman-induced
zation in nerve agent-damaged brain

al injury by the anticholinesterase
the carbamate carbofuran has been
ment, and oxidative stress [67]. The
p between an NO increase and a
n the CNS of rats exposed to these
finding to NO-induced impairment
to depletion of energy metabolites.
ngs of Damodaran *et al.* [46], who
levels of brain Acyl-CoA synthetase
mRNA levels of key mitochondria-
homa (BCL)-2-associated X protein
n killer protein (BOK). The authors
r cytochrome P-450 and generate
that would subsequently oxidize a

llular functions, mitochondria also
lease of mitochondrial proteins such
ctors. Members of the BCL-2 family
ptosis. Some of the BCL-2 family
ra large [BCL-XL], and BCL-2-like
o be anti-apoptotic, whereas other
death promoter [BAD], BAX, and
BAK]) induce apoptosis.
n of nerve agent poisoning has yet to
ntific data suggest that it involves

apoptotic neuronal cell death similar to that proposed following exposure to a micromolar concentration of L-glutamate [46]. Scientific support for an apoptotic mechanism of AChE-induced cell death was provided by Day and Greenfield [49]. They used a peptide derived from the C-terminus of AChE, which was homologous to β-amyloid, along with inhibitors of various cellular processes to trace the cell death pathway in organotypic hippocampal cultures and found that pathophysiological activity was produced via an apoptotic pathway. Their results indicated that the AChE peptide activated α7 nicotinic ACh receptor (α7 nAChR) and resulted in the activation of NMDA receptors, calcium release, mitochondrial dysfunction, and production of free radicals, leading to apoptotic cell death. They demonstrated that the activation of the caspase family is likely the key step in the apoptotic execution process, as they were able to block apoptotic cell death with caspase inhibitors. Day and Greenfield [49] state that similar conclusions have been drawn for β-amyloid toxicity and suggest that the induction of apoptotic cell death may be a common feature shared by neurotoxic β-sheet peptides.

Further, multiple genomic studies [39,40,46,48,52] have shown significant dysregulation of mitochondrial-associated genes, including several *Bcl-2*-related genes. For example, the study by Damodaran *et al.* [48] revealed overexpression of both anti-apoptotic (*Bcl-X*) and proapoptotic (*Bcl21II* and *caspase 6*) genes at 2 h following sarin exposure, indicating complex cell death/protection-related mechanisms soon after exposure.

The approach used by Pachiappan and colleagues [52] to investigate the mechanisms of nerve agent toxicity differed from the other transcription profiling studies of nerve agent exposure because they examined gene expression responses in human neuronal cells (SH-SY5Y cells), rather than in brain tissues, following exposure. In their study, they identified 224 genes whose expression was significantly altered by at least threefold following sarin exposure. Similar to the other genomic studies, they identified transcriptional changes related to dose and time of exposure. But unlike the other genomic studies, the authors identified a mitochondrial death pathway regulated by the transcription factor ETS2 as the main neurodegenerative signaling pathway activated in response to sarin. This unique finding is likely due to the use of a single cell type versus whole brain tissue where multiple cell types are represented.

In addition to the scientific data supporting an apoptotic mechanism of neuronal death, Baille *et al.* [25] also provide evidence for a necrotic mechanism of nerve agent-induced cell death within the first 24 h following a convulsant dose of soman and show very few apoptotic cells following the 24-h time period. They attribute the lack of apoptotic cells to a number of factors, including the rapid occurrence of apoptosis (suggesting that they would not see the apoptotic cells due to their elimination within a few hours). Another possibility for the lack of apoptotic cells is that apoptosis is

Material may be protected by copyright law (Title 17, U.S. Code)

an energy-dependent process, and since cellular energy is limited during nerve agent-induced excitotoxicity, cells may not have enough energy for apoptosis. The presence of an inflammatory response during nerve agent poisoning (discussed below) also supports a necrotic mechanism of nerve agent-induced cell death because inflammation is considered to be a key contributing factor to necrosis [68]. Therefore, nerve agent toxicity likely involves a form of programmed cell death with both apoptotic and necrotic characteristics.

6.4. Inflammation

Since the development of the model proposed by McDonough and Shih [11], many studies have shown that there is also an increase in proinflammatory cytokine mRNA and protein expression following nerve agent exposure that lasts hours to days later [38–40,46,48,53,69–74]. Further, there has been increasing evidence over the past several years implicating inflammatory reactions in the pathogenesis of several neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease, multiple sclerosis, and epilepsy [75,76]. Studies using various seizure models have shown an increase in cytokine mRNA and protein expression levels within 30 min following seizure induction in brain regions involved in seizure onset and spread [77–81]. Therefore, it is likely that the late phase of the model involves neuroinflammatory processes that lead to neuropathology following nerve agent exposure.

Spradling and colleagues recently analyzed sarin-induced gene expression changes in the rat amygdala, hippocampus, piriform cortex, septum, and thalamus [39,40], which are areas of the rat brain known to be affected by nerve agent-induced seizure [6,15,23,38]. A multitude of biological functions and canonical pathways were identified as being significantly altered following sarin-induced seizure. Many of the canonical pathways identified as being most significantly affected across all of the brain regions were indicative of an inflammatory response and included many proinflammatory cytokines such as interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α. These significantly altered pathways included ataxia telangiectasia-mutated protein (ATM) signaling, CD40 signaling, IL-10 signaling, IL-6 signaling, macrophage migration inhibitory factor (MIF) regulation of innate immunity, role of double-stranded RNA-activated protein kinase (PKR) in interferon induction and antiviral response, toll-like receptor signaling, and triggering receptor expressed on myeloid cells 1 (TREM1) signaling. Further, proinflammatory cytokines were among the top *de novo* networks identified as most significantly affected in all five brain regions from sarin-exposed seizing animals. Two of the top six networks were associated with an inflammatory response. One network of genes was centered on TNF-α as a central node and the other on IL-6 as a central node.

A significant increase in
early as 15 min following sari
response was still present at th
support of this finding, Dam
also observed an increase in
seizure activity. Damodaran
gene expression profiles imn
kines being among the signi
Chapman and colleagues [72]
IL-6, TNF-α, and prostaglan
at 2, 4, 6, 8, 24, 48, and 144
seizure. They observed a sigr
at their earliest time point of

Further support of a neur
exposure is provided by st
[69,71] previously showed i
following soman exposure. I
[70] used quantitative RT-I
response following a convul
[73] showed an increase of
molecule-1 (ICAM-1), and
mRNA in the whole cortex
soman exposure, which conf
where they saw an initial
exposure followed by an in
Johnson and Kan [74] have
cytokines in vulnerable bra
onset. They saw a significan
levels between 10 and 18 h a
et al. [53] used oligonucleoti
rat hippocampi at 1, 3, 6, 12
to a convulsant dose of sc
response to sarin [39,40], th
sion profiles over the first 24
frame, they identified a stro
immunological and inflamm
altered, and the top canonica
like receptor, IL-6, and IL-
studied the effects of soman
2), which is the initial enzym
tory prostaglandins (PGEs) a
initiation and propagation. T
sion and subsequent product
the rat brain from 4 h to 7 da

Material may be protected by copyright law (Title 17, U.S. Code)

ince cellular energy is limited during
cells may not have enough energy for
nmatory response during nerve agent
pports a necrotic mechanism of nerve
flammation is considered to be a key
Therefore, nerve agent toxicity likely
death with both apoptotic and necrotic

l proposed by McDonough and Shih
there is also an increase in proinflam-
ein expression following nerve agent
ter [38–40,46,48,53,69–74]. Further,
over the past several years implicating
ogenesis of several neurodegenerative
, Parkinson's disease, multiple sclerosis,
various seizure models have shown an
otein expression levels within 30 min
regions involved in seizure onset and
that the late phase of the model involves
ad to neuropathology following nerve

analyzed sarin-induced gene expression
campus, piriform cortex, septum, and
the rat brain known to be affected by
38]. A multitude of biological functions
ed as being significantly altered follow-
e canonical pathways identified as being
f the brain regions were indicative of an
nany proinflammatory cytokines such as
necrosis factor (TNF)-α. These signifi-
a telangiectasia-mutated protein (ATM)
ignaling, IL-6 signaling, macrophage
egulation of innate immunity, role of
tein kinase (PKR) in interferon induc-
eceptor signaling, and triggering recep-
1 (TREM1) signaling. Further,
ong the top *de novo* networks identified
five brain regions from sarin-exposed
ix networks were associated with an
of genes was centered on TNF-α as a
a central node.

A significant increase in proinflammatory gene expression was seen as early as 15 min following sarin-induced seizure onset, and this inflammatory response was still present at the latest observed time point of 24 h [39,40]. In support of this finding, Damodaran *et al.* [46,48] and Chapman *et al.* [72] also observed an increase in cytokine expression following sarin-induced seizure activity. Damodaran *et al.* [46,48] reported numerous changes in gene expression profiles immediately following sarin exposure with cytokines being among the significantly altered signal transduction pathways. Chapman and colleagues [72] monitored protein expression levels of IL-1β, IL-6, TNF-α, and prostaglandin E2 (PGE2) in the hippocampus and cortex at 2, 4, 6, 8, 24, 48, and 144 h, as well as 30 days, following sarin-induced seizure. They observed a significant increase in cytokine expression starting at their earliest time point of 2 h and peaking at 2–24 h following sarin.

Further support of a neuroinflammatory response following nerve agent exposure is provided by studies of soman intoxication. Svensson *et al.* [69,71] previously showed increases in IL-1β mRNA and protein levels following soman exposure. In addition, Dhote *et al.* [73] and Williams *et al.* [70] used quantitative RT-PCR to analyze the neuroinflammatory gene response following a convulsant dose of soman (1.6× LD_{50}). Dhote *et al.* [73] showed an increase of IL-1β, TNF-α, IL-6, intercellular adhesion molecule-1 (ICAM-1), and suppressor of cytokine signaling (SOCS) 3 mRNA in the whole cortex at 0.50, 1, 2, 6, 24, 48, and 168 h following soman exposure, which confirmed the earlier findings of Williams *et al.* [70] where they saw an initial upregulation of TNF-α mRNA at 2 h post-exposure followed by an increase in IL-1β and IL-6 mRNAs 6 h later. Johnson and Kan [74] have recently quantified the protein levels of these cytokines in vulnerable brain regions following soman-induced seizure onset. They saw a significant increase in IL-1β, IL-6, and TNF-α protein levels between 10 and 18 h after the peak mRNA expression levels. Dillman *et al.* [53] used oligonucleotide arrays to analyze gene expression profiles of rat hippocampi at 1, 3, 6, 12, 24, 48, 72, 96, and 168 h following exposure to a convulsant dose of soman. In agreement with the transcriptional response to sarin [39,40], they saw an increasing alteration in gene expression profiles over the first 24 h following soman exposure. Within this time frame, they identified a strong inflammatory response with the presence of immunological and inflammatory disease among the top biological processes altered, and the top canonical signaling pathways including p38 MAPK, toll-like receptor, IL-6, and IL-10. Angoa-Pérez and colleagues [19] recently studied the effects of soman on the expression of cyclooxygenase-2 (COX-2), which is the initial enzyme in the biosynthetic pathway of proinflammatory prostaglandins (PGEs) and a factor that has been implicated in seizure initiation and propagation. They found that the induction of COX-2 expression and subsequent production of PGEs correlated with seizure intensity in the rat brain from 4 h to 7 days, suggesting that these molecules could play a

Material may be protected by copyright law (Title 17, U.S. Code)

role in neuronal degeneration well after the cholinergic and glutamatergic response. Angoa-Pérez and colleagues hypothesize that seizures occurring in response to a PGE overload would likely not respond to the standard treatment of anticholinergics and benzodiazepines, indicating that other therapeutics, such as COX-2 inhibitors, should be added to prevent or minimize neuropathology that occurs in the later phase of the McDonough and Shih model [11].

Proinflammatory cytokines are known to mediate cellular communication and play a significant role in the pathological processes involved in various brain diseases, such as status epilepticus [75,76,78,81,82]. Although picomolar or low nanomolar ranges of cytokines enhance neuronal survival, higher concentrations have deleterious effects on neuronal viability [83]. Based on the findings of Spradling *et al.* and the findings of other investigators, inflammatory signaling pathways appear to be an important component of nerve agent-induced brain injury; however, the molecular mechanisms by which nerve agent-induced seizures produce acute neuroinflammation or how this phenomenon contributes to the ensuing neuropathology following exposure is still unclear. *In vitro* studies have shown that proinflammatory cytokines play a role in glutamate toxicity since they inhibit glial cells from taking up excess extracellular glutamate [84]. Cytokines are thought to further enhance this glutamatergic hyperactivity by increasing NMDA receptor activity [85], which promotes excitotoxic neuronal cell death [86,87].

6.5. BBB dysfunction

The blood–brain barrier (BBB) maintains the homeostasis of the CNS environment to ensure proper function [88]. Previous studies have shown that sarin [89] and soman [90–93] can cause breakdown of the BBB in sensitive areas such as the thalamus. Therefore, this disruption likely plays an important role in nerve agent-induced cell death in the sensitive brain regions. Further support of these findings was provided by Damodaran and colleagues [46,48], who identified numerous BBB-related genes that were altered at 15 min, 2 h, and 3 months following 0.5 and 1.0× LD_{50} sarin exposure. Their data identified multiple cell adhesion and cytoskeleton-related molecules altered at 15 min following sarin exposure, some of which were related to BBB function (neurexin 1-α; neurexin 1-β, PSD-95/SAP90-associated protein 4). Alteration in lipophilin mRNA levels at 15 min and 2 h indicated active degradation of CNS myelin. Lipophilin and myelin basic protein have been implicated for efficient axonal signal propagation and provide extrinsic trophic signals that affect the development and long-term survival of axons. The authors also noted the persistence of neurexin 1-β at 3 months, possibly indicating a continued dysfunctional BBB [46].

The mechanism by which nerve agents break down the BBB is still unknown. An increase in BBB permeability typically involves vesicular

transport and/or opening
Messent *et al.* [92] indicate
of endothelial vesicles, but
the endothelial tight junctio
an essential mechanism for
it should be noted that th
occur through very few dar
the small tissue sample se
Damodaran *et al.* [46], th
permeability to the early (1
cascades followed by tyrosi
upregulation of tyrosine ki
They speculated that this
junction permeability, wh
sodium-dependent neurotr
ters were also altered. Furt
primary mediator of injury
along with the changes in
such a scenario. They specu
processes, such as vasoactiv
may be involved in the bioc
vasoactive genes like bradyl

This scenario opens the
proinflammatory cytokines
brain is normally isolated fr
however, a neurotoxic insu
both a local and peripherall
has been seen in many ne
seizure [95], and studies hav
ing TNF-α, IL-1β, IL-6, an
BBB permeability [96–98].
of the BBB via disruption of
NO and matrix metallopro
gene response following sari
breakdown of the BBB as
ICAM1 and E-selectin in
septum, and thalamus. The
transduction cascades leadin
with the actin cytoskeleton,
peripheral leukocytes into
There was also a decrease in
main components of tight ju
integrity. The mechanism
opening of the BBB is still u

Material may be protected by copyright law (Title 17, U.S. Code)

er the cholinergic and glutamatergic
ypothesize that seizures occurring in
likely not respond to the standard
zodiazepines, indicating that other
ors, should be added to prevent or
n the later phase of the McDonough

n to mediate cellular communication
logical processes involved in various
75,76,78,81,82]. Although picomolar
s enhance neuronal survival, higher
on neuronal viability [83]. Based on
ndings of other investigators, inflam-
e an important component of nerve
he molecular mechanisms by which
cute neuroinflammation or how this
neuropathology following exposure
that proinflammatory cytokines play
hibit glial cells from taking up excess
are thought to further enhance this
ing NMDA receptor activity [85],
ell death [86,87].

ns the homeostasis of the CNS envi-
]. Previous studies have shown that
breakdown of the BBB in sensitive
is disruption likely plays an important
n the sensitive brain regions. Further
y Damodaran and colleagues [46,48],
genes that were altered at 15 min, 2 h,
× LD_{50} sarin exposure. Their data
toskeleton-related molecules altered
ome of which were related to BBB
SD-95/SAP90-associated protein 4).
at 15 min and 2 h indicated active
and myelin basic protein have been
pagation and provide extrinsic trophic
d long-term survival of axons. The
neurexin 1-β at 3 months, possibly
B [46].
agents break down the BBB is still
eability typically involves vesicular

transport and/or opening of tight junctions [94]. The findings of Grange-Messent *et al.* [92] indicate that soman-induced seizure increases the number of endothelial vesicles, but their data did not show any structural changes in the endothelial tight junctions, suggesting that tight-junction opening is not an essential mechanism for BBB dysfunction in soman poisoning. However, it should be noted that the authors point out that vascular leakage could occur through very few damaged tight junctions and may not be included in the small tissue sample selected for study. In the microarray study by Damodaran *et al.* [46], the authors attribute nerve agent-induced BBB permeability to the early (15 min) alteration of CAMKII and MAP kinase cascades followed by tyrosine phosphorylation, which was supported by the upregulation of tyrosine kinase receptor EHK-3 at 2 h post-exposure [48]. They speculated that this phosphorylation event might increase tight-junction permeability, which was supported by their observation that sodium-dependent neurotransmitter transporters and ATP-based transporters were also altered. Further, they point to the role of NO, which is a primary mediator of injury-induced BBB disruption. Induction of NOS-2 along with the changes in EDN3 (preproendothelin) expression indicates such a scenario. They speculate that agents involved in regulating vasoactive processes, such as vasoactive intestinal receptor-1 (VIPR1) and 2 (VIPR2), may be involved in the biochemical opening of the BBB, as shown for other vasoactive genes like bradykinin and angiotensin [46].

This scenario opens the door to another possible mechanism in which proinflammatory cytokines could contribute to nerve agent toxicity. The brain is normally isolated from the peripheral immune system via the BBB; however, a neurotoxic insult, such as a convulsant dose of sarin, can induce both a local and peripherally recruited inflammatory response. BBB damage has been seen in many neurodegenerative diseases and animal models of seizure [95], and studies have shown that proinflammatory cytokines including TNF-α, IL-1β, IL-6, and interferon-λ are implicated in the regulation of BBB permeability [96–98]. For example, IL-1β can affect the permeability of the BBB via disruption of the tight-junction organization or production of NO and matrix metalloproteinases in endothelial cells [98]. The observed gene response following sarin exposure [39,40] indicates that there could be a breakdown of the BBB as well. This was indicated by the upregulation of ICAM1 and E-selectin in the amygdala, hippocampus, piriform cortex, septum, and thalamus. These molecules are thought to be linked to signal transduction cascades leading to junctional reorganization as they can interact with the actin cytoskeleton, which in turn is an indicator of infiltration of peripheral leukocytes into damaged brain regions through the BBB [96]. There was also a decrease in occludin expression levels, which is one of the main components of tight junctions, indicating a possible loss of tight-junction integrity. The mechanism by which nerve agents cause seizure-related opening of the BBB is still unclear and requires further investigation.

Material may be protected by copyright law (Title 17, U.S. Code)

6.6. Repair and recovery

Victims of nerve agent poisoning often suffer long-term neurological deficits accompanied by brain neuronal cell death. Some survivors of the sarin Tokyo subway attack developed long-term chronic neurotoxicity characterized by CNS deficits and neurobehavioral impairments. Animal studies have also demonstrated that exposure to sarin caused brain neuronal cell death [89]. In the longer-term genomic studies of nerve agent exposure by Dillman *et al.* [53] and Damodaran *et al.* [46], it is apparent that nerve agent-induced transcriptional changes are still present weeks to months following exposure.

The data presented by Dillman and colleagues [53] provide insight into the temporal gene response of rat hippocampi following soman exposure. As discussed above, the significant biological processes and canonical pathways appeared to represent an inflammatory response during the first 24 h of the time course, which was also seen in the 24 h time course of sarin toxicity [39,40]. In the middle to late portions of the time course (24–96 h), Dillman and colleagues identified acute phase response pathway, complement system, and coagulation system as being significantly impacted by soman exposure 48–96 h post-exposure. The authors state that these findings are consistent with previous studies reporting the formation of gliotic scarring following soman exposure [99]. Collombet *et al.* [99] identified increases in GFAP and VEGF by immunohistochemistry in the hippocampi of soman-exposed mice during the time period of gliotic scar formation, which corresponded to the observed significant increases in GFAP and VEGF mRNA levels after soman exposure in the microarray study by Dillman *et al.* [53]. At the latest point of the time course (168 h), the significant biological processes and pathways appeared to follow the course of a recovery response, as suggested by biological processes such as RNA damage and repair, connective tissue development and function, and cell-to-cell signaling and interaction, and pathways such as glutamate metabolism, alanine and aspartate metabolism, D-glutamate metabolism, and D-glutamine metabolism. The authors used principal component analysis (PCA) to identify sources of relative variability among the hippocampal gene expression profiles (Figure 3). From their analysis, they identified exposure conditions (i.e., naive, vehicle-exposed, or soman-exposed) and post-exposure time point as the primary sources of variability in the dataset. The PCA in Figure 3 shows that sample groups for the 1, 3, 6, and 12-h time points partition progressively further away from the control sample group, while the 24, 72, 96, and 168-h sample groups partition progressively closer to the controls. Although these data suggest that gene expression profiles are returning to control levels, the three-dimensional plot indicates that none of the late time point groups partition with the control sample group, indicating altered gene expression at 168 h after soman exposure. Due to the similarities during the first 24 h of sarin [39,40] and soman [53] intoxication, we would anticipate a similar shift

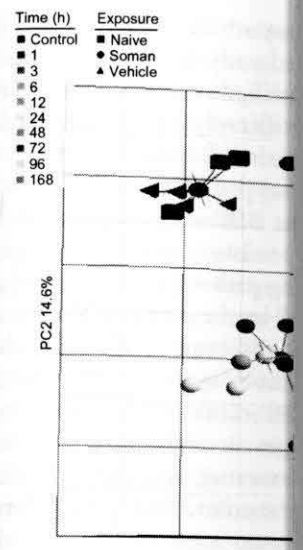


Figure 3 Principal compone
from soman-exposed rats ide
as primary sources of relati
isolated at the indicated tin
oligonucleotide microarray a
robust multiarray averaging
major sources of variability
represents the gene expressio
between any two points is a fi
Sample points that are near
expression profile, while san
large number of variables. P
color corresponds to the pos
group is shown as a sphere wi
connected by vectors to each
Naive controls were unexpo
soman. The principal compo
bility in gene expression leve
of the total information conte
of the variability in the data;
(*z*-axis) represents 12.2% of
dataset. Reprinted from Ref.

in gene expression involv
an injury and recovery p
studies analyzing gene e
needed to confirm this sa
seizure.

The study by Damod
present at 3 months follo

Material may be protected by copyright law (Title 17, U.S. Code)

n suffer long-term neurological deficits
ath. Some survivors of the sarin Tokyo
hronic neurotoxicity characterized by
npairments. Animal studies have also
used brain neuronal cell death [89]. In
ve agent exposure by Dillman *et al.* [53]
nt that nerve agent-induced transcrip-
months following exposure.
d colleagues [53] provide insight into
ppocampi following soman exposure.
ological processes and canonical path-
natory response during the first 24 h of
n the 24 h time course of sarin toxicity
of the time course (24–96 h), Dillman
response pathway, complement sys-
ng significantly impacted by soman
e authors state that these findings are
rting the formation of gliotic scarring
mbet *et al.* [99] identified increases in
emistry in the hippocampi of soman-
od of gliotic scar formation, which
cant increases in GFAP and VEGF
in the microarray study by Dillman
time course (168 h), the significant
eared to follow the course of a recov-
l processes such as RNA damage and
and function, and cell-to-cell signal-
as glutamate metabolism, alanine and
etabolism, and D-glutamine metabo-
mponent analysis (PCA) to identify
he hippocampal gene expression pro-
y identified exposure conditions (i.e.,
osed) and post-exposure time point as
e dataset. The PCA in Figure 3 shows
d 12-h time points partition progres-
mple group, while the 24, 72, 96, and
ively closer to the controls. Although
profiles are returning to control levels,
at none of the late time point groups
, indicating altered gene expression at
he similarities during the first 24 h of
on, we would anticipate a similar shift

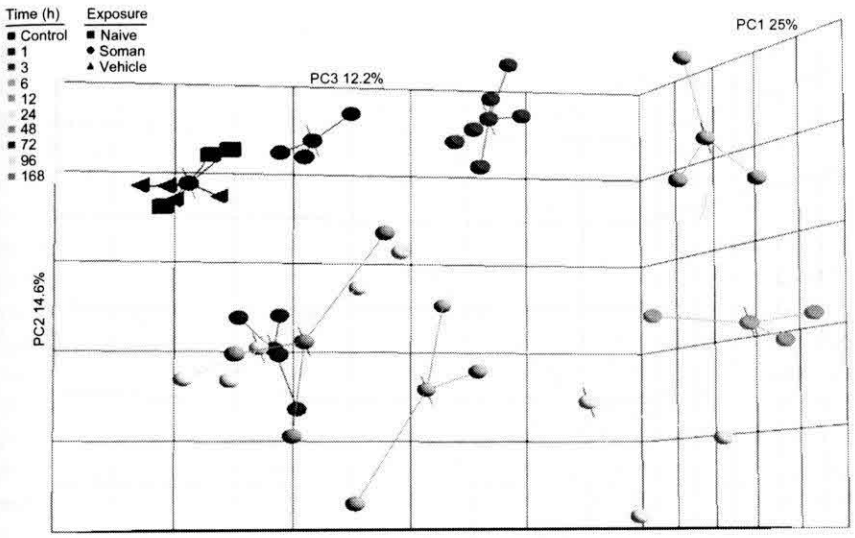


Figure 3 Principal component analysis (PCA) of hippocampal gene expression profiles from soman-exposed rats identifies exposure conditions and post-exposure time point as primary sources of relative variability in the dataset. Hippocampal tissues were isolated at the indicated time points following soman exposure and processed for oligonucleotide microarray analysis. The raw signal intensities were normalized using robust multiarray averaging (RMA) algorithm and visualized using PCA to identify major sources of variability in the data. Each point on the three-dimensional graph represents the gene expression profile of an individual biological sample. The distance between any two points is a function of the relative similarity between the two samples. Sample points that are near each other on the plot have a relatively similar gene expression profile, while sample points that are far apart are relatively different in a large number of variables. Point shape corresponds to exposure condition, and point color corresponds to the post-exposure time point. The centroid of each time point group is shown as a sphere with an angled black line through its center. Each centroid is connected by vectors to each of the sample points in its corresponding time point group. Naive controls were unexposed, and vehicle controls received all treatments except soman. The principal components in the three-dimensional graph represent the variability in gene expression levels seen within the dataset. The PCA plot rendered 51.8% of the total information content. Principal component 1 (PC1, *x*-axis) accounts for 25% of the variability in the data; PC2 (*y*-axis) represents 14.6% of the variability; and PC3 (*z*-axis) represents 12.2% of the variability in gene expression levels seen within the dataset. Reprinted from Ref. 53. Published 2009 American Chemical Society [53].

in gene expression involving molecular processes and pathways involved in an injury and recovery phase following sarin exposure. However, further studies analyzing gene expression profiles over a 168-h time period are needed to confirm this same mechanism of action following sarin-induced seizure.

The study by Damodaran *et al.* [46] detailed multiple gene alterations present at 3 months following sarin exposure. They noted the presence of

Material may be protected by copyright law (Title 17, U.S. Code)

ACCN1, SCN9A, ATPases, and ATP-based transporters, indicating that sarin-induced electrophysiological changes initiated early were minimized but did not completely return to normal. Genes classified as cytoskeletal and cell adhesion molecules were among the most significantly altered genes at 3 months after sarin exposure. β-Arrestin and neurexin1-β both maintained their altered state at 3 months. The authors state that the continued down-regulation of neurexin clearly supports the idea that BBB-related perturbations still persist at 3 months posttreatment, while persistent upregulation of β-arrestin confirms the continued aberrant signaling pathways mediated by G-protein-coupled receptors, which was evidenced by the continued over-expression of several classes of G-protein-coupled receptors (CAMKI and II, M2-AChR, GALR1, and CRLR). The authors speculate that the persistence of sarin-induced hyperphosphorylation causes defects in the tissue repair process. Therefore, long-term changes in certain molecules indicate that they likely play important roles in preserving, amplifying, and transmitting altered gene expression until a later time point, which could be either degenerative or regenerative in nature.

7. Future Directions in the Molecular Toxicology of Nerve Agents

The biological pathways and molecular mechanisms underlying nerve agent toxicity are still poorly understood. To help determine the molecular mechanisms of nerve agent toxicity, scientists have recently begun using "omics" technologies to assess the toxicological effects and physiological mechanisms of OP nerve agents. In a recent review [31], Everley and Dillman discuss how genomic and proteomic technologies have been used in over 50,000 research studies since the 1990s to help determine various biological processes, but there have been very few systems biology-related studies focusing on chemical warfare agents, especially OP nerve agents. In fact, there have been no large-scale proteomic studies of OP nerve agents. Previous, smaller-scale studies have shown that OP nerve agent exposure leads to the activation of many downstream proteins involved in numerous cellular signaling pathways regulated by protein phosphorylation [74,100]; however, additional studies need to be performed to investigate large-scale protein changes after nerve agent exposure. Also, in the study by Fauvelle *et al.* [18], the largest metabolomic study of nerve agent exposure to date, proton high-resolution magic angle spinning nuclear magnetic resonance spectroscopy (^{1}H HRMAS NMR) was used to describe the complex metabolic changes occurring in the piriform cortex and cerebellum of soman-exposed mice over a 7-day period. Using this global approach, the authors analyzed individual variation in the concentration of 13 metabolites.

As this technology matu
lomic studies involving
information regarding t
leading to cell/tissue da

The typical method
animal models. This app
and can be expensive
approach would involv
global molecular techr
method to rapidly assess
of any nerve agent. Alth
et al. [52], it should b
neuroblastoma cell line
mechanisms. Dissociate
closely resemble *in viv*
model system is well est
has previously been use
concern of using an *in vi*
features, such as the BB
propagation that faithfull
systems may offer some
models, including the al
pure populations of speci
vulnerability of a partic
prevents significant char
masked by the changes s
tissue. Moreover, the us
application of other mol
in vivo systems. For ex
posttranscriptional gene
system of choice in the b
gene function on a globa
analysis for genome-wid
identifying and validatin

8. Conclusion

The development
tists to look at the com
genes, proteins, and me
icants [27]. These techr
powerful approach in th

Material may be protected by copyright law (Title 17, U.S. Code)

TP-based transporters, indicating that
anges initiated early were minimized
al. Genes classified as cytoskeletal and
the most significantly altered genes at
stin and neurexin1-β both maintained
thors state that the continued down-
s the idea that BBB-related perturba-
ment, while persistent upregulation of
rrant signaling pathways mediated by
was evidenced by the continued over-
tein-coupled receptors (CAMKI and
R). The authors speculate that the
osphorylation causes defects in the
g-term changes in certain molecules
t roles in preserving, amplifying, and
ntil a later time point, which could be
nature.

THE MOLECULAR
AGENTS

lecular mechanisms underlying nerve
od. To help determine the molecular
scientists have recently begun using
xicological effects and physiological
a recent review [31], Everley and
oteomic technologies have been used
the 1990s to help determine various
een very few systems biology-related
agents, especially OP nerve agents. In
roteomic studies of OP nerve agents.
hown that OP nerve agent exposure
tream proteins involved in numerous
by protein phosphorylation [74,100];
e performed to investigate large-scale
osure. Also, in the study by Fauvelle
udy of nerve agent exposure to date,
pinning nuclear magnetic resonance
was used to describe the complex
piriform cortex and cerebellum of
riod. Using this global approach, the
n the concentration of 13 metabolites.

As this technology matures, there should be a growing number of metabolomic studies involving nerve agents. These studies will provide valuable information regarding the molecular mechanisms of nerve agent toxicity leading to cell/tissue damage and long-term neurobehavioral deficits.

The typical methodology for studying nerve agents involves the use of animal models. This approach requires specialized facilities and support staff and can be expensive and time-consuming for large studies. Another approach would involve the use of an *in vitro* model system coupled with global molecular techniques to establish a high-throughput screening method to rapidly assess the molecular targets and toxicological mechanisms of any nerve agent. Although this approach was recently used by Pachiappan *et al.* [52], it should be noted that their study was performed using a neuroblastoma cell line (SH-SY5Y), which may not truly represent *in vivo* mechanisms. Dissociated primary nerve cell cultures, however, may more closely resemble *in vivo* biology than immortalized neuronal cells. This model system is well established in the field of neuroscience [101–104] and has previously been used to evaluate neurotoxicant effects [103]. A major concern of using an *in vitro* system involves the lack of important functional features, such as the BBB, heterogeneous cell–cell interactions, and seizure propagation that faithfully mimics what is observed *in vivo*. However, *in vitro* systems may offer some advantages for neurotoxicity assessment over *in vivo* models, including the ability to study the molecular effects of toxicants in pure populations of specific cell types. This capability not only highlights the vulnerability of a particular cell type (e.g., neurons or glial cells) but also prevents significant changes in one cell type from being "diluted out" or masked by the changes seen in a complex mixture of cells, such as in whole tissue. Moreover, the use of an *in vitro* model system would easily allow the application of other molecular technologies that are not currently feasible in *in vivo* systems. For example, RNA interference (RNAi), a method of posttranscriptional gene silencing, has rapidly become the high-throughput system of choice in the biotechnology industry for mechanistically assessing gene function on a global scale [104]. This technique paired with microarray analysis for genome-wide expression profiling has proved to be effective in identifying and validating molecular mechanisms [105].

8. CONCLUSIONS

The development of global molecular technologies has enabled scientists to look at the complete complement, expression, and regulation of genes, proteins, and metabolites involved in the response to various toxicants [27]. These techniques coupled with pathway modeling provide a powerful approach in the development of neuroprotectants against nerve

Material may be protected by copyright law (Title 17, U.S. Code)

agent exposure since they identify potential biomarkers of exposure and targets for therapeutic intervention. Because current countermeasures may not fully prevent neurological damage, particularly in scenarios where treatment is delayed (e.g., civilian terrorist attack), this type of in-depth analysis is critical to examine the molecular effects following nerve agent exposure and to identify therapeutics that can reduce or block the cascade of secondary events that lead to neuropathology and associated functional impairments.

A large amount of data obtained from scientific studies utilizing global molecular techniques support the temporal model proposed by McDonough and Shih [11] as well as provide further evidence for the presence of non-cholinergic and non-glutamatergic systems during the late phase response to nerve agent poisoning (Figure 4), which was first suggested from studies using AChE knockout mice [106]. These studies showed that acute nerve agent exposure alters the expression of multiple genes and pathways at an early time point (0.25–2 h) following exposure, suggesting concurrent activation of protective mechanisms as well as neurodegenerative changes [39,40,46,48,53]. These data also suggest that the persistence and magnitude of early gene expression changes predict long-term pathological developments [46].

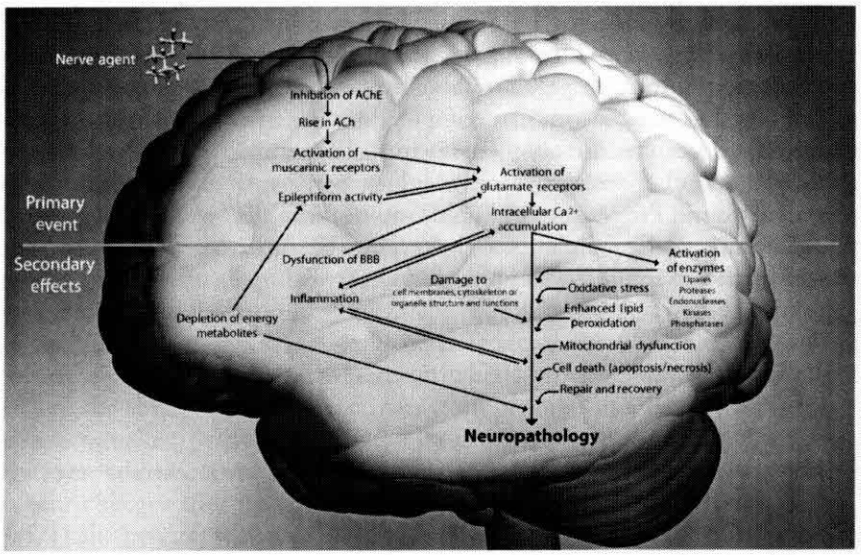
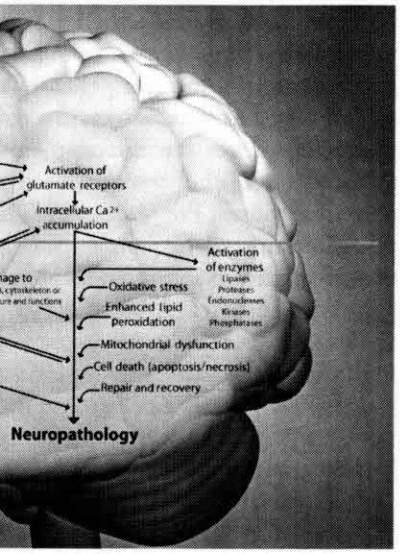


Figure 4 Overview of the neurotransmitter and nonneurotransmitter systems that are impacted by nerve agent intoxication. Data from a variety of sources, as described in the text, suggest cholinergic and non-cholinergic mechanisms involved in downstream sequelae after nerve agent exposure, leading to neuropathology. Illustration by Alexandre Katos.

Material may be protected by copyright law (Title 17, U.S. Code)

tential biomarkers of exposure and
ecause current countermeasures may
ge, particularly in scenarios where
rorist attack), this type of in-depth
ecular effects following nerve agent
at can reduce or block the cascade of
athology and associated functional

om scientific studies utilizing global
temporal model proposed by
s provide further evidence for the
-glutamatergic systems during the
isoning (Figure 4), which was first
nockout mice [106]. These studies
re alters the expression of multiple
oint (0.25–2 h) following exposure,
otective mechanisms as well as neu-
3]. These data also suggest that the
e expression changes predict long-



and nonneurotransmitter systems that are
m a variety of sources, as described in the
ic mechanisms involved in downstream
ing to neuropathology. Illustration by

Mitochondria are proposed to play a significant role in seizure generation in certain forms of epilepsy, and multiple genomic studies [39,40,46,48,52] have shown significant dysregulation of mitochondrial-associated genes, including several BCL-2-related genes, following nerve agent exposure. Therefore, these mitochondrial genes should be considered as promising targets for future therapeutic strategies to treat nerve agent poisoning. Also, the rapid and persistent alteration of proinflammatory cytokines seen in these studies strongly suggests that they may play a causal role in long-term pathological changes following exposure to OP nerve agents. Therefore, antagonism of proinflammatory molecules, as well as their receptors and signaling pathways, may represent a novel approach for the development of drug therapies that would reduce neurological damage when given after the onset of seizures and secondary responses that lead to brain injury.

Data obtained from these global molecular assays have increased our understanding of the molecular response to nerve agent exposure during the acute toxic response as well as during the neurodegenerative and recovery processes [53]. The findings of Dillman *et al.* [53] show a gradual but incomplete return to control gene expression profiles at 168 h following soman exposure, but the extent of recovery at the molecular level over a longer time-frame (weeks or months) is uncertain. Even though the gene expression levels appear to be returning to normal after exposure, cell death and/or neuronal rewiring that occurred during upregulation may be irreversible [46,53].

Gene expression profiling has provided insight into the mechanisms of nerve agent-induced brain injury by identifying the initial molecular responses as well as secondary responses to nerve agent poisoning. However, determining the exact sequence of events is difficult due to the temporal overlap of many of the identified processes and pathways. The use of global profiling techniques will further accelerate our understanding of nerve agent toxicity and help determine the mechanisms of toxicity. Large-scale proteomic and metabolomic studies using nerve agents have been lacking. Just as microarrays have been used to map signaling networks for sarin and soman, these techniques could also complement and expand on that work to identify additional signaling pathways impacting nerve agent toxicity [31]. Further, these "omic" techniques could be applied to other nerve agents (such as tabun and VX) to determine if these agents have molecular effects similar to those of sarin and soman. The use of global molecular profiling techniques, such as genomics, proteomics, and metabolomics, will further accelerate our understanding of the molecular mechanisms involved in nerve agent-induced toxicity and identify therapeutics that can reduce or block the cascade of secondary events that lead to neuropathology and associated functional impairments.

Material may be protected by copyright law (Title 17, U.S. Code)

ACKNOWLEDGMENTS

We thank Mr. Alexandre Katos for assistance with Figure 1 and graphic design of Figures 2 and 4; and Drs. Albert Ruff, John McDonough, and Tsung-ming Shih for critical reading of the chapter. Dr. Spradling was supported by the CounterACT Program, National Institutes of Health Office of the Director and the National Institute of Allergy and Infectious Diseases (OD# Y1-OD-9613-01; Dr. Tsung-Ming Shih, PI). Dr. Dillman was supported by the CounterACT Program, National Institutes of Health Office of the Director and the National Institute of Allergy and Infectious Diseases (OD# Y1-OD-9613-01; Dr. Tsung-Ming Shih, PI) and the Defense Threat Reduction Agency-Joint Science and Technology Office, Medical S&T Division.

Disclaimer. The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

REFERENCES

[1] H. Salem, A.L. Ternay Jr., J.K. Smart, Brief history and use of chemical warfare agents in warfare and terrorism, in: J.A. Romano Jr., B.J. Lukey, H. Salem (Eds.), Chemical Warfare Agents: Chemistry, Pharmacology, Toxicology, and Therapeutics, CRC Press-Taylor & Francis Group, LLC, Boca Raton, FL, 2008, pp. 1–20.

[2] M.A. Brown, K.A. Brix, Review of health consequences from high-, intermediate and low-level exposure to organophosphorus nerve agents, J. Appl. Toxicol. 18 (1998) 393–408.

[3] F. Schmaltz, Neurosciences and research on chemical weapons of mass destruction in Nazi Germany, J. Hist. Neurosci. 15 (2006) 186–209.

[4] A. Hoffman, A. Eisenkraft, A. Finkelstein, O. Schein, E. Rotman, T. Dushnitsky, A decade after the Tokyo sarin attack: A review of neurological follow-up of the victims, Mil. Med. 172 (2007) 607–610.

[5] Y. Loh, M.M. Swanberg, M.V. Ingram, J. Newmark, Case report: long-term cognitive sequelae of sarin exposure, Neurotoxicology 31 (2010) 244–246.

[6] V. Aroniadou-Anderjaska, T.H. Figueiredo, J.P. Apland, F. Qashu, M.F.M. Braga, Primary brain targets of nerve agents: The role of the amygdala in comparison to the hippocampus, Neurotoxicology 30 (2009) 772–776.

[7] N.B. Munro, K.R. Ambrose, A.P. Watson, Toxicity of the organophosphate chemical warfare agents GA, GB, and VX: Implications for public protection, Environ. Health Perspect. 102 (1994) 18–38.

[8] T. Martin, S. Lobert, Chemical warfare: Toxicity of nerve agents, Crit. Care Nurse 23 (2003) 15–20.

[9] F. Worek, L. Szinicz, P. Eyer, H. Thiermann, Evaluation of oxime efficacy in nerve agent poisoning: Development of a kinetic-based dynamic model, Toxicol. Appl. Pharmacol. 209 (2005) 193–202.

[10] M.A. Dunn, B.E. Hackley, F.R. Sidell, Pretreatment for nerve agent exposure, in: F.R. Sidell, E.T. Takafuji, D.R. Franz (Eds.), Textbook of Military Medicine: Medical Aspects of Chemical and Biological Warfare, Borden Institute, Walter Reed Army Medical Center, Washington, DC, 1997, pp. 181–196.

[11] J.H. McDonough Jr., T.M. Shih, Neuropharmacological mechanisms of nerve agent-induced seizure and neuropathology, Neurosci. Biobehav. Rev. 21 (1997) 559–579.

[12] J. Bajgar, Complex view on poisoning with nerve agents and organophosphates, Acta Med. (Hradec Králové) 48 (2005) 3–21.

[13] M. Filbert, E. Levine
damage by blocking
Radiol. Def. 3 (2005)

[14] T. Myhrer, S. Enger,
soman-induced seizu
184–191.

[15] T. Myhrer, Neuronal
caused by nerve agen
1–14.

[16] J.H. McDonough Jr.
seizures, Neurosci. Bi

[17] P. Carpentier, A. Foc
J.M. Kamenka, G. B
ethal effects of the ph
ogy 15 (1994) 837–8

[18] F. Fauvelle, F. Dora
Demilly, P. Arvers,
convulsive dose of so
99–111.

[19] M. Angoa-Pérez, C
J.H. McDonough, D
link between seizures
738–746.

[20] J. Bajgar, L. Ševelová,
ical and behavioral ef
(2004) 497–507.

[21] T. Myhrer, S. Enger,
in seizure induction i

[22] M.J.A. Joosen, E. J
P.J. Lucassen, H.P.M
reduced neurogenesis

[23] J.H. McDonough Jr.,
nerve agent induced r
anti-convulsant actio

[24] T.M. Shih, S.M. Du
seizures is critical for
(2003) 69–80.

[25] V. Baille, P.G.H.
G. Lallement, P. C
revisited, Toxicology

[26] O.U. Scremin, T.M.
neurologic and beha
J. Pharmacol. Exp. T

[27] J. Vlaanderen, L.E.
N. Rothman, R. V
and environmental h
Med. 67 (2010) 136–

[28] M. Schena, D. Shalo
expression patterns
467–470.

[29] C.A. Afshari, Persp
Endocrinology 143

Material may be protected by copyright law (Title 17, U.S. Code)

with Figure 1 and graphic design of Figures 2
h, and Tsung-ming Shih for critical reading of
ne CounterACT Program, National Institutes
nal Institute of Allergy and Infectious Diseases
hih, PI). Dr. Dillman was supported by the
Health Office of the Director and the National
D# Y1-OD-9613-01; Dr. Tsung-Ming Shih,
ency-Joint Science and Technology Office,

ed herein are the private views of the authors
eflecting the views of the Department of the

rief history and use of chemical warfare agents
no Jr., B.J. Lukey, H. Salem (Eds.), Chemical
ology, Toxicology, and Therapeutics, CRC
oca Raton, FL, 2008, pp. 1–20.
ealth consequences from high-, intermediate
sphorus nerve agents, J. Appl. Toxicol. 18

n on chemical weapons of mass destruction in
006) 186–209.
in, O. Schein, E. Rotman, T. Dushnitsky, A
A review of neurological follow-up of the
.
, J. Newmark, Case report: long-term cogni-
oxicology 31 (2010) 244–246.
redo, J.P. Apland, F. Qashu, M.F.M. Braga,
he role of the amygdala in comparison to the
09) 772–776.
son, Toxicity of the organophosphate chemi-
Implications for public protection, Environ.

Toxicity of nerve agents, Crit. Care Nurse 23

rmann, Evaluation of oxime efficacy in nerve
netic-based dynamic model, Toxicol. Appl.

, Pretreatment for nerve agent exposure, in:
nz (Eds.), Textbook of Military Medicine:
ological Warfare, Borden Institute, Walter
on, DC, 1997, pp. 181–196.
pharmacological mechanisms of nerve agent-
eurosci. Biobehav. Rev. 21 (1997) 559–579.
vith nerve agents and organophosphates, Acta

[13] M. Filbert, E. Levine, G. Ballough, Neuroprotection for nerve agent-induced brain damage by blocking delayed calcium overload: A review, J. Med. Chem. Biol. Radiol. Def. 3 (2005) 1–21.

[14] T. Myhrer, S. Enger, P. Aas, Efficacy of immediate and subsequent therapies against soman-induced seizures and lethality in rats, Basic Clin. Pharmacol. 98 (2006) 184–191.

[15] T. Myhrer, Neuronal structures involved in the induction and propagation of seizures caused by nerve agents: Implications for medical treatment, Toxicology 239 (2007) 1–14.

[16] J.H. McDonough Jr., T.M. Shih, Pharmacological modulation of soman-induced seizures, Neurosci. Biobehav. Rev. 17 (1993) 203–215.

[17] P. Carpentier, A. Foquin Tarricone, N. Bodjarian, G. Rondouin, M. Lerner Natoli, J.M. Kamenka, G. Blanchet, M. Denoyer, G. Lallement, Anticonvulsant and antilethal effects of the phencyclidine derivative TCP in soman poisoning, Neurotoxicology 15 (1994) 837–852.

[18] F. Fauvelle, F. Dorandeu, P. Carpentier, A. Foquin, H. Rabeson, D. Graveron-Demilly, P. Arvers, G. Testylier, Changes in mouse brain metabolism following a convulsive dose of soman: A proton HRMAS NMR study, Toxicology 267 (2010) 99–111.

[19] M. Angoa-Pérez, C.W. Kreipke, D.M. Thomas, K.E. Van Shura, M. Lyman, J.H. McDonough, D.M. Kuhn, Soman increases neuronal COX-2 levels: Possible link between seizures and protracted neuronal damage, Neurotoxicology 31 (2010) 738–746.

[20] J. Bajgar, L. Ševelová, G. Krejčová, J. Fusek, J. Vachek, J. Kassa, J. Herink, Biochemical and behavioral effects of soman vapors in low concentrations, Inhal. Toxicol. 16 (2004) 497–507.

[21] T. Myhrer, S. Enger, P. Aas, Anticonvulsant effects of damage to structures involved in seizure induction in rats exposed to soman, Neurotoxicology 28 (2007) 819–828.

[22] M.J.A. Joosen, E. Jousma, T.M. van den Boom, W.C. Kuijpers, A.B. Smit, P.J. Lucassen, H.P.M. van Helden, Long-term cognitive deficits accompanied by reduced neurogenesis after soman poisoning, Neurotoxicology 30 (2009) 72–80.

[23] J.H. McDonough Jr., L.W. Dochterman, C.D. Smith, T.M. Shih, Protection against nerve agent induced neuropathology, but not cardiac pathology, is associated with the anti-convulsant action of drug treatment, Neurotoxicology 15 (1995) 123–132.

[24] T.M. Shih, S.M. Duniho, J.H. McDonough Jr., Control of nerve agent-induced seizures is critical for neuroprotection and survival, Toxicol. Appl. Pharmacol. 188 (2003) 69–80.

[25] V. Baille, P.G.H. Clarke, G. Brochier, F. Dorandeu, J.M. Verna, E. Four, G. Lallement, P. Carpentier, Soman-induced convulsions: The neuropathology revisited, Toxicology 15 (2005) 1–24.

[26] O.U. Scremin, T.M. Shih, L. Huynh, M. Roch, R. Booth, D.J. Jenden, Delayed neurologic and behavioral effects of subtoxic doses of cholinesterase inhibitors, J. Pharmacol. Exp. Ther. 304 (2003) 1111–1119.

[27] J. Vlaanderen, L.E. Moore, M.T. Smith, Q. Lan, L. Zhang, C.F. Skibola, N. Rothman, R. Vermeulen, Application of omics technologies in occupational and environmental health research; current status and projection, Occup. Environ. Med. 67 (2010) 136–143.

[28] M. Schena, D. Shalon, R.W. Davis, P.O. Brown, Quantitative monitoring of gene expression patterns with complementary DNA microarray, Science 270 (1995) 467–470.

[29] C.A. Afshari, Perspective: Microarray technology, seeing more than spots, Endocrinology 143 (2002) 1983–1989.

Material may be protected by copyright law (Title 17, U.S. Code)

[30] J.F. Hocquette, I. Cassar-Malek, A. Scalbert, F. Guillou, Contribution of genomics to the understanding of physiological functions, J. Physiol. Pharmacol. 60 (Suppl. 3) (2009) 5–16.
[31] P.A. Everley, J.F. Dillman III, Genomics and proteomics in chemical warfare agent research: Recent studies and future applications, Toxicol. Lett. 198 (2010) 297–303.
[32] K. Aggarwal, L.H. Choe, K.H. Lee, Shotgun proteomics using the iTRAQ isobaric tags, Brief. Funct. Genomic Proteomic 5 (2006) 112–120.
[33] T. Liu, J. Hu, H. Li, iTRAQ-based shotgun neuroproteomics, Methods Mol. Biol. 566 (2009) 201–216.
[34] J.V. Olsen, B. Blagoev, F. Gnad, B. Macek, C. Kumar, P. Mortensen, M. Mann, Global, in vivo, and site-specific phosphorylation dynamics in signaling networks, Cell 127 (2006) 635–648.
[35] D.A. Hall, J. Ptacek, M. Snyder, Protein microarray technology, Mech. Ageing Dev. 128 (2007) 161–167.
[36] J.C. Lindon, E. Holmes, J.K. Nicholson, Metabonomics techniques and applications to pharmaceutical research and development, Pharm. Res. 23 (2006) 1075–1088.
[37] J.F. Dillman III, A.I. Hege, C.S. Phillips, L.D. Orzolek, A.J. Sylvester, C. Bossone, C. Henemyre-Harris, R.C. Kiser, Y.W. Choi, J.J. Schlager, C.L. Sabourin, Microarray analysis of mouse ear tissue exposed to bis-(2-chloroethyl) sulfide: Gene expression profiles correlate with treatment efficacy and an established clinical endpoint, J. Pharmacol. Exp. Ther. 317 (2006) 76–87.
[38] L.A. Zimmer, M. Ennis, M.T. Shipley, Soman-induced seizures rapidly activate astrocytes and microglia in discrete brain regions, J. Comp. Neurol. 378 (1997) 482–492.
[39] K.D. Spradling, L.A. Lumley, C.L. Robison, J.L. Meyerhoff, J.F. Dillman, III, Genomic analysis of piriform cortex following exposure to the organophosphonate anticholinesterase sarin reveals gene expression profile differences correlated with seizure induction, submitted for publication (copy on file with author).
[40] K.D. Spradling, L.A. Lumley, C.L. Robison, J.L. Meyerhoff, J.F. Dillman, III, Transcriptional response of the nerve agent-sensitive regions amygdala, hippocampus, piriform cortex, septum, and thalamus to the anticholinesterase sarin, submitted for publication (copy on file with author).
[41] T.M. Shih, Time course effects of soman on acetylcholine and choline levels in six discrete areas of the rat brain, Psychopharmacology (Berlin) 78 (1982) 170–175.
[42] G. Lallement, P. Carpentier, A. Collet, D. Baubichon, I. Pernot Marino, G. Blanchet, Extracellular acetylcholine changes in rat limbic structures during soman-induced seizure, Neurotoxicology 13 (1992) 557–568.
[43] G. Lallement, P. Carpentier, A. Collet, I. Pernot Marino, D. Baubichon, G. Blanchet, Effects of soman-induced seizures on different extracellular amino acid levels and on glutamate uptake in rat hippocampus, Brain Res. 563 (1991) 234–240.
[44] C.J. Flynn, L. Wecker, Elevated choline levels in brain: A non-cholinergic component of organophosphate toxicity, Biochem. Pharmacol. 35 (1986) 3115–3121.
[45] M.J. Berridge, Unlocking the secrets of cell signaling, Annu. Rev. Physiol. 67 (2005) 1–21.
[46] T.V. Damodaran, A.G. Patel, S.T. Greenfield, H.K. Dressman, S.M. Lin, M.B. Abou-Donia, Gene expression profiles of the rat brain both immediately and 3 months following acute sarin exposure, Biochem. Pharmacol. 71 (2006) 497–520.
[47] D. Weinshenker, P. Szot, The role of catecholamines in seizure susceptibility: New results using genetically engineered mice, Pharmacol. Therapeut. 94 (2002) 213–233.
[48] T.V. Damodaran, S.T. Greenfield, A.G. Patel, H.K. Dressman, S.K. Lin, M. B. Abou-Donia, Toxicogenomic studies of the rat brain at an early time point following acute sarin exposure, Neurochem. Res. 31 (2006) 367–381.

[49] T. Day, S.A. Greenfield,
nal cell death: Character
334–342.
[50] C. Cerella, M. Diederic
stressor in cell damage, d
[51] J.L. Blanton, J.A. D'Am
expression patterns of R
mice, J. Biochem. Mol.
[52] A. Pachiappan, M.M.
V. Sivakumar, P. Gopal
pathway of human neur
dose sarin (GB), Chem.
[53] J.F. Dillman III, C.S.
R.K. Kan, Gene express
acetylcholinesterase inhi
[54] T.L. Pazdernik, M.R.
induced seizures: Limbi
J. Appl. Toxicol. 21 (20
[55] A.W. Abu-Qare, M.B.
mine bromide increase
deoxyguanosine, biomar
[56] M.B. Abou-Donia, A.M
Sensorimotor deficit and
mine bromide and sarin
[57] M.J. Barrett, V. Alones
derived oxidative stress i
(2004) 420–429.
[58] B. Kalmar, L. Greensmi
oxidative stress, Adv. Dr
[59] V. Baille-Le Crom, J.M.
G. Rondouin, G. Laller
HSP70 in hippocampus
J. Neurosci. Res. 45 (19
[60] G. Zsurka, W.S. Kunz,
epileptic phenotypes, J. I
[61] A.P. Kudin, D. Malinska,
homogenates of brain ti
inhibitors, Biochim. Bio
[62] D. Malinska, B. Kulawi
A. Szewczyk, W.S. Kun
mitochondria contribute
1797 (2010) 1163–1170.
[63] A.P. Kudin, N.Y.B. Bin
terization of superoxide-
279 (2004) 4127–4135.
[64] A.P. Kudin, T.A. Kudina
Seizure-dependent mod
hippocampus, Eur. J. Ne
[65] R.C. Jovic, Correlation
rats poisoned by soman,
[66] T.L. Pazdernik, R.S. Cr
cerebral glucose utilizatio

Material may be protected by copyright law (Title 17, U.S. Code)

bert, F. Guillou, Contribution of genomics to
ctions, J. Physiol. Pharmacol. 60 (Suppl. 3)

cs and proteomics in chemical warfare agent
lications, Toxicol. Lett. 198 (2010) 297–303.
otgun proteomics using the iTRAQ isobaric
5 (2006) 112–120.
otgun neuroproteomics, Methods Mol. Biol.

Macek, C. Kumar, P. Mortensen, M. Mann,
phorylation dynamics in signaling networks,

n microarray technology, Mech. Ageing Dev.

n, Metabonomics techniques and applications
ment, Pharm. Res. 23 (2006) 1075–1088.
os, L.D. Orzolek, A.J. Sylvester, C. Bossone,
. Choi, J.J. Schlager, C.L. Sabourin, Micro-
d to bis-(2-chloroethyl) sulfide: Gene expres-
efficacy and an established clinical endpoint,
–87.
Soman-induced seizures rapidly activate astro-
ons, J. Comp. Neurol. 378 (1997) 482–492.
Robison, J.L. Meyerhoff, J.F. Dillman, III,
llowing exposure to the organophosphonate
xpression profile differences correlated with
tion (copy on file with author).
Robison, J.L. Meyerhoff, J.F. Dillman, III,
ent-sensitive regions amygdala, hippocampus,
to the anticholinesterase sarin, submitted for

an on acetylcholine and choline levels in six
armacology (Berlin) 78 (1982) 170–175.
D. Baubichon, I. Pernot Marino, G. Blanchet,
rat limbic structures during soman-induced
7–568.
. Pernot Marino, D. Baubichon, G. Blanchet,
fferent extracellular amino acid levels and on
Brain Res. 563 (1991) 234–240.
e levels in brain: A non-cholinergic compo-
hem. Pharmacol. 35 (1986) 3115–3121.
cell signaling, Annu. Rev. Physiol. 67 (2005)

Greenfield, H.K. Dressman, S.M. Lin,
profiles of the rat brain both immediately
exposure, Biochem. Pharmacol. 71 (2006)

catecholamines in seizure susceptibility: New
e, Pharmacol. Therapeut. 94 (2002) 213–233.
A.G. Patel, H.K. Dressman, S.K. Lin, M.
ies of the rat brain at an early time point
hem. Res. 31 (2006) 367–381.

[49] T. Day, S.A. Greenfield, A peptide derived from acetylcholinesterase induces neuronal cell death: Characterisation of possible mechanisms, Exp. Brain Res. 153 (2003) 334–342.

[50] C. Cerella, M. Diederich, L. Ghibelli, The dual role of calcium as messenger and stressor in cell damage, death, and survival, Int. J. Cell Biol. 2010 (2010) 1–14.

[51] J.L. Blanton, J.A. D'Ambrozio, J.E. Sistrunk, E.G. Midboe, Global changes in the expression patterns of RNA isolated from the hippocampus and cortex of VX exposed mice, J. Biochem. Mol. Toxicol. 18 (2004) 115–123.

[52] A. Pachiappan, M.M. Thwin, L. Weng Keong, F.K. Lee, J. Manikandan, V. Sivakumar, P. Gopalakrishnakone, ETS2 regulating neurodegenerative signaling pathway of human neuronal (SH-SY5Y) cells exposed to single and repeated low-dose sarin (GB), Chem. Res. Toxicol. 22 (2009) 990–996.

[53] J.F. Dillman III, C.S. Phillips, D.M. Kniffin, C.P. Tompkins, T.A. Hamilton, R.K. Kan, Gene expression profiling of rat hippocampus following exposure to the acetylcholinesterase inhibitor soman, Chem. Res. Toxicol. 22 (2009) 633–638.

[54] T.L. Pazdernik, M.R. Emerson, R. Cross, S.R. Nelson, F.E. Samson, Soman-induced seizures: Limbic activity, oxidative stress and neuroprotective proteins, J. Appl. Toxicol. 21 (2001) S87–S94.

[55] A.W. Abu-Qare, M.B. Abou-Donia, Combined exposure to sarin and pyridostigmine bromide increased levels of rat urinary 3-nitrotyrosine and 8-hydroxy-2′-deoxyguanosine, biomarkers of oxidative stress, Toxicol. Lett. 123 (2001) 51–58.

[56] M.B. Abou-Donia, A.M. Dechkovskaia, L.B. Goldstein, S.L. Bullman, W.A. Khan, Sensorimotor deficit and cholinergic changes following coexposure with pyridostigmine bromide and sarin in rats, Toxicol. Sci. 66 (2002) 148–158.

[57] M.J. Barrett, V. Alones, K.X. Wang, L. Phan, R.H. Swerdlow, Mitochondria-derived oxidative stress induces a heat shock protein response, J. Neurosci. Res. 78 (2004) 420–429.

[58] B. Kalmar, L. Greensmith, Induction of heat shock proteins for protection against oxidative stress, Adv. Drug Deliv. Rev. 61 (2009) 310–318.

[59] V. Baille-Le Crom, J.M. Collombet, M.F. Burckhart, A. Foquin, I. Pernot-Marino, G. Rondouin, G. Lallement, Time course and regional expression of C-FOS and HSP70 in hippocampus and piriform cortex following soman-induced seizures, J. Neurosci. Res. 45 (1996) 513–524.

[60] G. Zsurka, W.S. Kunz, Mitochondrial dysfunction in neurological disorders with epileptic phenotypes, J. Bioenerg. Biomembr. 42(6) (2010) 443–448.

[61] A.P. Kudin, D. Malinska, W.S. Kunz, Sites of generation of reactive oxygen species in homogenates of brain tissue determined with the use of respiratory substrates and inhibitors, Biochim. Biophys. Acta 1777 (2008) 689–695.

[62] D. Malinska, B. Kulawiak, A.P. Kudin, R. Kovacs, C. Huchzermeyer, O. Kann, A. Szewczyk, W.S. Kunz, Complex III-dependent superoxide production of brain mitochondria contributes to seizure-related ROS formation, Biochim. Biophys. Acta 1797 (2010) 1163–1170.

[63] A.P. Kudin, N.Y.B. Bimpong-Buta, S. Vielhaber, C.E. Elger, W.S. Kunz, Characterization of superoxide-producing sites in isolated brain mitochondria, J. Biol. Chem. 279 (2004) 4127–4135.

[64] A.P. Kudin, T.A. Kudina, J. Seyfried, S. Vielhaber, H. Beck, C.E. Elger, W.S. Kunz, Seizure-dependent modulation of mitochondrial oxidative phosphorylation in rat hippocampus, Eur. J. Neurosci. 15 (2002) 1105–1114.

[65] R.C. Jovic, Correlation between signs of toxicity and some biochemical changes in rats poisoned by soman, Eur. J. Pharmacol. 25 (1974) 159–164.

[66] T.L. Pazdernik, R.S. Cross, M. Giesler, F.E. Samson, S.R. Nelson, Changes in local cerebral glucose utilization induced by convulsants, Neuroscience 14 (1985) 823–835.

Material may be protected by copyright law (Title 17, U.S. Code)

[67] R.C. Gupta, D. Milatovic, W.D. Dettbarn, Depletion of energy metabolites following acetylcholinesterase inhibitor-induced status epilepticus: Protection by antioxidants, Neurotoxicology 22 (2001) 271–282.
[68] P.G.H. Clarke, Apoptosis versus necrosis-how valid a dichotomy for neurons? in: V.E. Koliatsos, R.R. Ratan (Eds.), Cell Death and Diseases of the Nervous System, Humana Press, Inc., Totowa, NJ, 1999, pp. 3–28.
[69] I. Svensson, L. Waara, L. Johansson, A. Bucht, G. Cassel, Soman-induced interleukin-1β mRNA and protein in rat brain, Neurotoxicology 22 (2001) 355–362.
[70] A.J. Williams, R. Berti, C. Yao, R.A. Price, L.C. Velarde, I. Koplovitz, S.M. Schultz, F.C. Tortella, J.R. Dave, Central neuro-inflammatory gene response following soman exposure in the rat, Neurosci. Lett. 349 (2003) 147–150.
[71] I. Svensson, L. Waara, G. Cassel, Effects of HI 6, diazepam and atropine on soman-induced IL-1β protein in rat brain, Neurotoxicology 26 (2005) 173–181.
[72] S. Chapman, T. Kadar, E. Gilat, Seizure duration following sarin exposure affects neuro-inflammatory markers in the rat brain, Neurotoxicology 27 (2006) 277–283.
[73] F. Dhote, A. Peinnequin, P. Carpentier, V. Baille, C. Delacour, A. Foquin, G. Lallement, F. Dorandeu, Prolonged inflammatory gene response following soman-induced seizures in mice, Toxicology 238 (2007) 166–176.
[74] E.A. Johnson, R.K. Kan, The acute phase response and soman-induced status epilepticus: Temporal and regional changes in rat brain cytokine concentrations, J. Neuroinflamm. 7 (2010) 1–9.
[75] A. Vezzani, Inflammation and epilepsy, Epilepsy Curr. 5 (2005) 1–6.
[76] J. Choi, S. Koh, Role of brain inflammation in epileptogenesis, Yonsei Med. J. 49 (2008) 1–18.
[77] M. Minami, Y. Kuraishi, M. Satoh, Effects of kainic acid on messenger RNA levels of IL-1 beta, IL-6, TNF alpha and LIF in the rat brain, Biochem. Biophys. Res. Commun. 176 (1991) 593–598.
[78] M.G. De Simoni, C. Perego, T. Ravizza, D. Moneta, M. Conti, F. Marchesi, A. De Luigi, S. Garattini, A. Vezzani, Inflammatory cytokines and related genes are induced in the rat hippocampus by limbic status epilepticus, Eur. J. Neurosci. 12 (2000) 2623–2633.
[79] K.A. Lehtimaki, J. Peltola, E. Koskikallio, T. Keranen, J. Honkaniemi, Expression of cytokines and cytokine receptors in the rat brain after kainic acid-induced seizures, Mol. Brain Res. 110 (2003) 253–260.
[80] M. Oprica, C. Riksson, M. Schultzberg, Inflammatory mechanisms associated with brain damage induced by kainic acid with special reference to the interleukin-1 system, J. Cell. Mol. Med. 7 (2003) 127–140.
[81] B. Voutsinos-Porche, E. Koning, H. Kaplan, A. Ferrandon, M. Guenounou, A. Nehlig, J. Motte, Temporal patterns of the cerebral inflammatory response in the rat lithium-pilocarpine model of temporal lobe epilepsy, Neurobiol. Dis. 17 (2004) 385–402.
[82] B. Viviani, S. Bartesaghi, E. Corsini, C.L. Galli, M. Marinovich, Cytokines role in neurodegenerative events, Toxicol. Lett. 149 (2004) 85–89.
[83] M.C. Morganti-Kossmann, T. Kossmann, S.M. Wahl, Cytokines and neuropathology, Trends Pharmacol. Sci. 13 (1992) 286–291.
[84] Z.C. Ye, H. Sontheimer, Cytokine modulation of glial glutamate uptake: A possible involvement of nitric oxide, Neuroreport 7 (1996) 2181–2185.
[85] B. Viviani, S. Bartesaghi, F. Gardoni, A. Vezzani, M.M. Behrens, T. Bartfai, M. Binaglia, E. Corsini, M. Di Luca, C.L. Galli, M. Marinovich, Interleukin-1β enhances NMDA receptor-mediated intracellular calcium increase through activation of the Src family of kinases, J. Neurosci. 23 (2003) 8692–8700.

[86] T. Ravizza, S.M. Luca
J.C.R. Randle, S. Allan
novel anticonvulsive stra
[87] T. Ravizza, B. Gagliar
adaptive immunity duri
experimental models an
142–160.
[88] F. Joo, Endothelial cells
differences, Prog. Neur
[89] A. Abdel-Rahman, A.K.
blood brain barrier per
brain: Dose-response rel
[90] Y. Ashani, G.N. Catrava
brain barrier following
Pharmacol. 30 (1981) 2
[91] P. Carpentier, I.S. Dela
related opening of the
with the acute neurop
(1990) 293–299.
[92] V. Grange-Messent,
P. Carpentier, Seizure-
anticholinesterase comp
Biol. 45 (1999) 1–14.
[93] J.P. Petrali, D.M. Maxw
rat blood-brain barrier,
[94] G.E. Palade, Blood cap
368–384.
[95] P.M. Carvey, B. Hende
tive disease: A rhetorical
[96] S.J. Bolton, D.C. Anth
and zonula occludens-
induced blood-brain bar
[97] W.G. Mayhan, Cellula
disruption of the blood-
[98] S.M. Allan, P.J. Tyrrell,
5 (2005) 629–640.
[99] J.M. Collombet, E. Fou
D. Baubichon, G. Lalle
angiogenesis in damage
[100] L.N. Bottalico, G.D. M
campus following son
March 16–20, 2008, Se
[101] P.G. Nelson, Neurona
Perspect. 26 (1978) 125
[102] C.A. Tyson, N.H. Stac
(Ed.), In Vitro Toxicity
Inc., New York, 1992,
[103] R.F.M. Silva, A.S. Falc
ciate primary nerve cell
Lett. 163 (2006) 1–9.

Material may be protected by copyright law (Title 17, U.S. Code)

barn, Depletion of energy metabolites follow-
ced status epilepticus: Protection by antioxi-
-282.
osis-how valid a dichotomy for neurons? in:
ll Death and Diseases of the Nervous System,
, pp. 3–28.
. Bucht, G. Cassel, Soman-induced interleu-
n, Neurotoxicology 22 (2001) 355–362.
Price, L.C. Velarde, I. Koplovitz, S.M. Schultz,
euro-inflammatory gene response following
ett. 349 (2003) 147–150.
ts of HI 6, diazepam and atropine on soman-
urotoxicology 26 (2005) 173–181.
ure duration following sarin exposure affects
brain, Neurotoxicology 27 (2006) 277–283.
ntier, V. Baille, C. Delacour, A. Foquin,
ged inflammatory gene response following
ology 238 (2007) 166–176.
hase response and soman-induced status epi-
nges in rat brain cytokine concentrations,

, Epilepsy Curr. 5 (2005) 1–6.
nation in epileptogenesis, Yonsei Med. J. 49

cts of kainic acid on messenger RNA levels of
in the rat brain, Biochem. Biophys. Res.

za, D. Moneta, M. Conti, F. Marchesi, A. De
atory cytokines and related genes are induced
tus epilepticus, Eur. J. Neurosci. 12 (2000)

io, T. Keranen, J. Honkaniemi, Expression of
e rat brain after kainic acid-induced seizures,

g, Inflammatory mechanisms associated with
with special reference to the interleukin-1
7–140.
lan, A. Ferrandon, M. Guenounou, A. Nehlig,
bral inflammatory response in the rat lithium-
psy, Neurobiol. Dis. 17 (2004) 385–402.
.L. Galli, M. Marinovich, Cytokines role in
t. 149 (2004) 85–89.
n, S.M. Wahl, Cytokines and neuropathology,
91.
dulation of glial glutamate uptake: A possible
rt 7 (1996) 2181–2185.
i, A. Vezzani, M.M. Behrens, T. Bartfai,
C.L. Galli, M. Marinovich, Interleukin-1β
racellular calcium increase through activation
. 23 (2003) 8692–8700.

[86] T. Ravizza, S.M. Lucas, S. Balosso, L. Bernardino, G. Ku, F. Noé, J. Malva, J.C.R. Randle, S. Allan, A. Vezzani, Inactivation of caspase-1 in rodent brain: A novel anticonvulsive strategy, Epilepsia 47 (2006) 1160–1168.

[87] T. Ravizza, B. Gagliardi, F. Noé, K. Boer, E. Aronica, A. Vezzani, Innate and adaptive immunity during epileptogenesis and spontaneous seizures: Evidence from experimental models and human temporal lobe epilepsy, Neurobiol. Dis. 29 (2008) 142–160.

[88] F. Joo, Endothelial cells of the brain and other organ systems: Some similarities and differences, Prog. Neurobiol. 48 (1996) 255–273.

[89] A. Abdel-Rahman, A.K. Shetty, M.B. Abou-Donia, Acute exposure to sarin increases blood brain barrier permeability and induces neuropathological changes in the rat brain: Dose-response relationships, Neuroscience 113 (2002) 721–741.

[90] Y. Ashani, G.N. Catravas, Seizure-induced changes in the permeability of the blood-brain barrier following administration of anticholinesterase drugs to rat, Biochem. Pharmacol. 30 (1981) 2593–2601.

[91] P. Carpentier, I.S. Delamanche, M. Le Bert, G. Blanchet, C. Bouchaud, Seizure-related opening of the blood-brain barrier induced by soman: Possible correlation with the acute neuropathology observed in poisoned rats, Neurotoxicology 541 (1990) 293–299.

[92] V. Grange-Messent, C. Bouchaud, M. Jamme, G. Lallement, A. Foquin, P. Carpentier, Seizure-related opening of the blood-brain barrier produced by the anticholinesterase compound, soman: New ultrastructural observations, Cell. Mol. Biol. 45 (1999) 1–14.

[93] J.P. Petrali, D.M. Maxwell, D.E. Lenz, K.R. Mills, A study of the effects of soman on rat blood-brain barrier, Anat. Rec. 211 (1991) 351–352.

[94] G.E. Palade, Blood capillaries of the heart and other organs, Circulation 24 (1961) 368–384.

[95] P.M. Carvey, B. Hendey, A.J. Monahan, The blood-brain barrier in neurodegenerative disease: A rhetorical perspective, J. Neurochem. 111 (2009) 291–314.

[96] S.J. Bolton, D.C. Anthony, V.H. Perry, Loss of the tight junction proteins occludin and zonula occludens-1 from cerebral vascular endothelium during neutrophil-induced blood-brain barrier breakdown in vivo, Neuroscience 86 (1998) 1245–1257.

[97] W.G. Mayhan, Cellular mechanisms by which tumor necrosis factor-α produces disruption of the blood-brain barrier, Brain Res. 927 (2002) 144–152.

[98] S.M. Allan, P.J. Tyrrell, N.J. Rothwell, Interleukin-1 and neuronal injury, Nat. Rev. 5 (2005) 629–640.

[99] J.M. Collombet, E. Four, W. Fauquette, M.F. Burckhart, C. Masqueliez, D. Bernabé, D. Baubichon, G. Lallement, Soman poisoning induces delayed astrogliotic scar and angiogenesis in damaged mouse brain areas, Neurotoxicology 28 (2007) 38–48.

[100] L.N. Bottalico, G.D. Minsavage, J.F. Dillman, III, Proteomic analysis of rat hippocampus following soman exposure. Society of Toxicology Annual Meeting, March 16–20, 2008, Seattle, WA, Abst 153.

[101] P.G. Nelson, Neuronal cell cultures as toxicologic test systems, Environ. Health Perspect. 26 (1978) 125–133.

[102] C.A. Tyson, N.H. Stacey, In vitro technology, trends, and issues, in: J.M. Frazier (Ed.), In Vitro Toxicity Testing: Applications to Safety Evaluation, Marcel Dekker, Inc., New York, 1992, pp. 13–43.

[103] R.F.M. Silva, A.S. Falcão, A. Fernandes, A.C. Gordo, M.A. Brito, D. Brites, Dissociate primary nerve cell cultures as models for assessment of neurotoxicity, Toxicol. Lett. 163 (2006) 1–9.

Material may be protected by copyright law (Title 17, U.S. Code)

[104] V. Mittal, Improving the efficiency of RNA interference in mammals, Nat. Rev. Genet. 5 (2004) 355–365.
[105] P. Stålberg, M. Santesson, S. Ekeblad, M.H. Lejonklou, B. Skogseid, Recognizing genes differentially regulated in vitro by the multiple endocrine neoplasia type I (MEN1) gene, using RNA interference and oligonucleotide microarrays, Surgery 140 (2006) 921–931.
[106] E.G. Duysen, B. Li, W. Xie, L.M. Schopfer, R.S. Anderson, C.A. Broomfield, O. Lockridge, Evidence for nonacetylcholinesterase targets of organophosphorus nerve agent: Supersensitivity of acetylcholinesterase knockout mouse to VX lethality, J. Pharmacol. Exp. Ther. 299 (2001) 528–535.

CHAPTER F

TOXICITY O NANOPART

Masanori Horie[1]

Contents

1. Introduction
2. Oxidative Stress
   2.1. Introductio
   2.2. Inducement oxide nano
   2.3. Biological e
3. Apoptosis
4. Inflammation Re
5. Long-Term Effect
6. Biological Effect
   6.1. Particle size
   6.2. Surface are
   6.3. Solubility
   6.4. Adsorption
   6.5. Zeta potenti
   6.6. Other factor
7. Methods of Evalu Nanoparticles
8. Gene Expression Manufactured Na
   8.1. Application profiling an pulmonary s
   8.2. *In vivo* stud
   8.3. *In vivo* DNA nanomateria

[1] Health Research Institute, Natio Osaka, Japan
[2] Research Institute of Science fo
*Corresponding author. Tel.: +8
E-mail address: masa-horie@aist.go

Advances in Molecular Toxicology, V
ISSN 1872-0854, DOI: 10.1016/

Material may be protected by copyright law (Title 17, U.S. Code)